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THE CAMBIUM AND ITS DERIVATIVE TISSUES
II. SIZE VARIATIONS OF CAMBIAL INITIALS IN GYMNOSPERMS AND ANGIOSPERMS

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INTRODUCTION

Much has been written during the last fifty years concerning the relations between cell size, and body size, nuclear size, chromosomal number, and chromosomal mass. One group of botanists and zoologists, including such classical writers as Sachs (1893), Driesch (1898, 1900), and Boveri (1904), maintain that the size of the cells in specific organs or organisms remains constant regardless of variations in growth or stature, whereas another group hold that cell number rather than cell size is fixed. A second controversy revolves around the question whether the nucleo-cytoplasmic relation is a constant or a self-regulating ratio, and, more recently, whether dwarf and giant mutants are produced by changes in the number or in the size of chromosomes.

Many of the discrepancies in the conclusions of these writers appear to be due to an intensive study of a particular tissue, organism, or stage in ontogeny without reference to what may occur in other tissues, organisms, or developmental stages. Levi (1906) has shown that in mammals the size variations of epithelial and gland cells—elements which continue to divide throughout life—are insignificant, whereas such highly differentiated cells as nerve fibers, lens fibers, muscle fibers, and ganglion cells tend to be considerably larger in large animals than in small ones. Thus, the necessity for *extensive* preliminary, comparative investigations in selecting material for *intensive* experimental research, and to serve as checks upon excessive generalization from limited induction, is well illustrated by the literature dealing with body size and cell size.

In the first investigation of this series[1] an attempt was made to determine, by means of an extensive reconnaissance survey, what are some of the more fundamental types of size variations that occur in the tracheary

[1] Bailey, I. W., and Tupper, W. W. Size variation in tracheary cells: I. A comparison between the secondary xylems of vascular cryptogams, gymnosperms and angiosperms. Proc. Amer. Acad. Arts and Sci. 54: 149–204. 1918.

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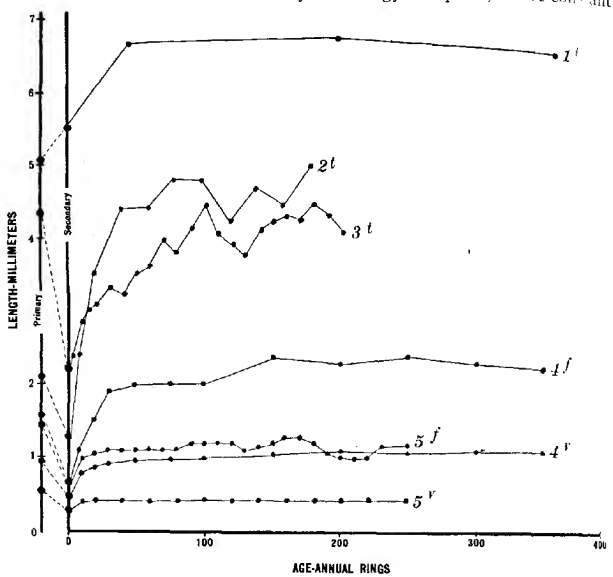
cells of the secondary xylem of vascular plants. The elements were found to fluctuate considerably in length in different parts of an organ or plant, in individuals grown under different environmental conditions, and in different groups of the Siphonogama. As shown in text figure 1, the average length of the tracheary cells, in a given radius and at a particular height in the stem of an arborescent dicotyledon or gymnosperm, is not constant

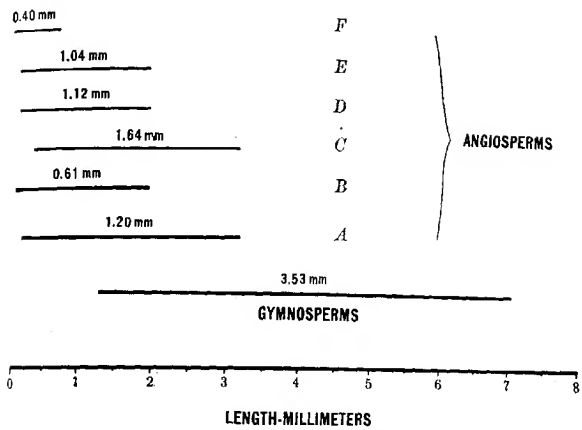


TEXT FIG. 1. Curves showing variations in average length of tracheary cells in passing from the innermost to the outermost secondary xylem of the stem. Average lengths of primary tracheary elements shown for comparison. 1, cycad; 2, conifer; 3, vesselless dicotyledon; 4, dicotyledon with primitive vessels; 5, dicotyledon with highly differentiated vessels. *t*, tracheids; *f*, fiber tracheids; *v*, vessel-segments. Modified from Bailey and Tupper.

in succeeding annual rings, but tends to increase rapidly for a period of years and subsequently to fluctuate more or less above and below a certain general level. This length-on-age curve varies in different portions of the stem and in plants grown under different environmental influences. In normal forest trees, its crest tends to be higher in the "clear length" of the stem and lower in the crown, in the stump, and in proximity to burls, severe injuries, and other disturbing factors. Although these somatic variations, due to physiological and ecological factors, are so varied and extensive as to render

difficult the isolation of germinal fluctuations in a limited number of closely related plants, the study of a wide series of Siphonogamia reveals striking differences in the size of the tracheary cells in different groups of plants. For example, the average length of the tracheids in the outer rings of the secondary xylem of 152 gymnosperms was 3.53 ± 0.07 mm. (SD = 1.25 ± 0.05 mm.); whereas in comparable material of 275 dicotyledons, from 31 orders and 118 families, the mean length of the fiber tracheids[2] and vessel-segments was 1.20 ± 0.02 mm. (SD = 0.50 ± 0.01 mm.) and 0.61 ± 0.02 mm. (SD = 0.41 ± 0.01 mm.) respectively (text fig. 2).

The reduced length of the tracheary elements in the secondary xylem of



TEXT FIG. 2. Limits of variability of average lengths of tracheids in the older wood of 152 gymnosperms contrasted with the limits of variability of (*A*) average lengths of fiber tracheids in older wood of 275 miscellaneous dicotyledons, (*B*) average lengths of vessel-segments in 275 miscellaneous dicotyls, (*C*) average lengths of fiber tracheids in older wood of 53 dicotyls having primitive vessels, (*D*) average lengths of vessel-segments in 53 primitive dicotyls, (*E*) average lengths of fiber tracheids in older wood of 109 dicotyls having highly specialized vessels, and (*F*) average lengths of vessel segments in 109 specialized dicotyls. Mean of average lengths shown numerically.

dicotyledons appears to be closely correlated with the development and differentiation of vessels. This is indicated, not only by the striking general contrast between the sizes of the tracheary elements in plants which have vessels (Gnetales, dicotyledons) and in those which are devoid of them (vascular cryptogams, gymnosperms, vesselless Trochodendraceae, and

[2] Using this term in a general sense to include tracheids, fiber tracheids, libriform fibers, and septate fibers, but excluding substitute fibers.

Magnoliaceae, text fig. 1), but also by the fact that the tracheary cells in the dicotyledons tend to shorten as the vessels become more and more highly specialized (text fig. 2).[3]

In all of the arborescent dicotyledons and gymnosperms, with the probable exception of the Cordaitales, Bennettitales, and Cycadales, the first formed tracheary cells of the secondary xylem are relatively small and are considerably shorter than the adjoining elements of the primary xylem (text fig. 1). This is in marked contrast to the conditions which appear to have prevailed in the stems of many of the lower vascular plants. In forms having relatively wide zones of primary wood, the innermost secondary tracheids resembled in size the outermost primary tracheids. It seems probable that in the evolution of the higher gymnosperms and dicotyledons, with reduction in the amount of primary xylem and with other changes in the innermost portion of the stele, there has been a concomitant shortening of the first formed elements of the secondary xylem.

The size of the cells in the secondary xylem is determined by (1) the size of the cambial initials, and by (2) changes that take place in their derivative cells during differentiation into tracheary elements. It is conceivable, therefore, that the variations in the size of the tracheary elements may be closely correlated with similar fluctuations in the size of the meristematic cells. It is also conceivable, however, that the cells of the lateral meristem are of relatively uniform size, as hypothesized by Strasburger (1893), Winkler (1916), and others, and that the differences in the size of tracheary cells are due entirely to changes, *e.g.*, expansion, division, etc., which occur during differentiation of the xylem. The present paper is devoted to a comparative study of the size variations of cambial initials and tracheary cells.

Material and Methods

There are two methods of determining the sizes of the cells in a given tissue: by measurements taken (1) from sections and (2) from macerations. Each method has certain inherent advantages and disadvantages. In macerations it is possible to isolate individual cells and measure their various dimensions, but it is necessary to allow for differences in breakage.

[3] The secondary xylem of the Calamariales, Sphenophyllales, Lepidophytinae, Cycadofilices, and Gymnospermae, exclusive of the Gnetales, is comparatively homogeneous and its tracheary cells are of a single generalized type, so-called tracheids. In the Gnetales and Dicotyledoneae specialization or "division of labor" appears to have occurred among these cells. Certain vertical series of tracheids have become modified and function principally in conducting liquids, whereas others have assumed a mechanical rôle. As the vessels of the dicotyledons become more and more highly differentiated, their segments change their shape and structure and lose their resemblance to tracheids. At the same time, the surrounding tracheary elements tend to take on a more fiber-like structure, their pits becoming vestigial by the gradual disappearance of the bordering areas in the secondary walls.

shrinkage or contraction, etc. Of course, it is difficult to macerate the cambium and other soft tissues. The average length of vertically elongated elements may be obtained with a considerable degree of accuracy from longitudinal, tangential sections of tissues in which the elements are arranged in regular radial rows, *i.e.*, as in the cambium or xylem of gymnosperms. The lengths of the fiber tracheids and vessel-segments in most dicotyledons have to be obtained from macerations.

The measurements of the cells of conifers, recorded in the following table, were obtained from serial, tangential, longitudinal sections of the cambium and adjacent xylem, and were checked by measurements taken from macerations. In the case of the dicotyledons, the tabulated values were secured from tangential sections of the cambium and macerations of the outermost layer of the underlying xylem. The means are averages of fifty measurements, and their probable errors vary between 0.005 and 0.05 mm.

It is evident from these data that in Ginkgo and the Coniferae the length of the cambial initials closely resembles, but usually is slightly less than,[1] that of the tracheids of the last formed growth layer of the xylem. In the dicotyledons, on the other hand, the meristematic cells are in most cases considerably shorter than the fiber tracheids, but are of approximately the same length as the vessel-segments. However, they tend to be slightly shorter than the vessel-segments in species (Abies, Euptelea, Myristica, Liquidambar, Rhizophora, Nyssa) having primitive types of vessels, and a little longer than these cells in plants having highly specialized conducting systems. Therefore, by allowing for a 5–10 percent error, it is possible to use the tracheids of gymnosperms and the vessel-segments of arborescent and fruticose dicotyledons as indexes of the approximate length of the cambial initials in these two important groups of the vascular plants.

The principal types of size (length) variations that occur in the tracheary cells of the secondary xylem are closely paralleled by similar fundamental fluctuations in the longitudinal dimension of cambial initials. Thus, these meristematic cells vary in different parts of a plant or organ, in individuals grown under different environmental conditions, and in different groups of the Siphonogama. They are relatively short in young shoots and twigs of Gingko and Coniferae, but during subsequent growth increase in length for a period of years until a certain size level has been attained, after which they fluctuate more or less in response to various physiological and environmental influences. In comparable material, the normal length-on-age curve for the cambial initials tends to be considerably lower and flatter in the dicotyledons than in the conifers, and in plants having highly differentiated vessels[2] than in those in which the conducting systems are relatively primitive (text fig. 3, page 363).

[1] Mischke's (1890) calculations of elongation are based upon an erroneous premise, as has been pointed out by Klinken (1914).

[2] In certain highly specialized dicotyledons the length of the short cambial initials, vessel-segments, and substitute fibers may remain constant or nearly constant during successive increases in the circumference of the stem, as suggested by Sanio (1873–74).

TABLE I.

Comparative Lengths of Tracheary and Meristematic Cells

GYMNOSPERMAE

	Cambial Initials			Tracheids		
	Max.	Mean	Min.	Max.	Mean	Min.
I. GINKGOALES						
1. Ginkgoaceae						
[4] *Ginkgo biloba* L.	3.0	2.2	1.4	2.9	2.2	1.4
II. CONIFERAE						
2. Taxaceae						
[6] *Taxus cuspidata* Sieb. and Zucc.	1.6	1.1	0.8	1.7	1.3	0.8
3. Pinaceae						
(*a*) Abieteae						
Pinus Strobus L.	4.0	3.2	2.3	4.6	3.4	2.2
Picea Abies (L.) Karst.	4.2	3.3	2.4	4.2	3.0	2.8
Larix decidua Mill.	5.0	4.0	2.5	5.4	4.2	2.7
[6] *Pseudotsuga taxifolia* (Lamb.) Britton	1.6	1.2	0.7	1.8	1.2	0.9
[6] *Abies Nordmanniana* Spach.	1.5	1.1	0.7	1.8	1.2	1.0
[6] *Cedrus libani* Barrel	2.6	2.0	1.2	2.7	2.1	1.3
[6] *Tsuga canadensis* (L.) Carr.	1.8	1.4	0.9	2.1	1.5	1.1
(*b*) Taxodieae						
[6] *Sciadopitys verticillata* Sieb. and Zucc.	1.6	1.2	0.7	1.6	1.3	1.0
[6] *Sequoia gigantea* Lindl. and Gord.	4.5	3.7	2.5	4.5	3.8	2.8
(*c*) Cupressineae						
[6] *Thuja occidentalis* L.	2.1	1.5	0.7	2.4	1.7	1.3
Juniperus virginiana L.	3.0	2.2	1.0	3.0	2.3	1.4

ANGIOSPERMAE-DICOTYLEDONEAE[7]

	Vessel-segments			Cambial Initials			Fiber Tracheids		
	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.
A. ARCHICHLAMYDEAE									
I. SALICALES									
1. Salicaceae									
Populus sp.	0.70	0.50	0.19	0.66	0.49	0.35	1.19	0.90	0.61
II. JUGLANDALES									
2. Juglandaceae									
Carya glabra Sweet	0.63	0.43	0.20	0.70	0.56	0.40	1.69	1.13	0.65
Carya ovata (Mill.) C. Koch	0.55	0.51	0.47	0.60	0.52	0.42	1.69	1.30	0.96
III. FAGALES									
3. Betulaceae									
Alnus incana (L.) Moench	0.84	0.66	0.43	0.72	0.60	0.34	1.20	1.89	0.56
Betula populifolia Marsh.	1.17	0.89	0.65	1.16	0.94	0.70	1.60	1.31	0.91
4. Fagaceae									
Quercus alba L.	0.60	0.46	0.36	0.67	0.53	0.39	1.42	1.00	0.80
IV. URTICALES									
5. Ulmaceae									
Ulmus americana L.	0.59	0.33	0.21	0.47	0.35	0.27	1.96	1.53	1.12
V. RANALES									
6. Trochodendraceae									
Eupteleu polyandra Sieb. and Zucc.	0.97	0.72	0.39	0.86	0.63	0.40	1.42	0.95	0.59
7. Annonaceae									
Annona reticulata L.	0.43	0.29	0.13	0.39	0.33	0.22	1.71	1.28	0.85

[6] Material obtained from small branches or young stems.

[7] Material obtained from stems of various ages.

TABLE I (*Continued*)

	Vessel-segments			Cambial Initials			Ray Initials		
	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.
Phaeanthus ebracteolatus Merr.	0.58	0.39	0.23	0.61	0.44	0.27	1.40	0.94	0.61
8. Myristicaceae									
Myristica philippensis Lam.	1.64	1.42	0.84	1.62	1.31	0.99	2.00	1.60	1.12
9. Lauraceae									
Litsea glutinosa C. R. Rob.	0.74	0.52	0.36	0.70	0.55	0.39	1.49	0.95	0.50
Sassafras officinale Nees and Eberm.	0.50	0.39	0.22	0.50	0.30	0.27	0.83	0.61	0.38
VI. ROSALES									
10. Pittosporaceae									
Pittosporum pentandrum (Blanco) Merr.	0.90	0.66	0.29	1.01	0.80	0.56	1.22	0.99	0.70
11. Hamamelidaceae									
Liquidambar Styraciflua L.	1.39	0.76	0.41	0.98	0.70	0.40	1.75	0.96	0.67
12. Rosaceae									
Pyrus Malus L.	0.72	0.51	0.29	0.74	0.53	0.34	1.29	0.98	0.61
Prunus serotina Ehrh.	0.58	0.45	0.23	0.50	0.40	0.32	1.40	0.99	0.58
Pyrus sp.	0.80	0.57	0.44	0.77	0.66	0.52	1.17	0.92	0.50
13. Leguminosae									
Robinia Pseudo-Acacia L.	0.22	0.18	0.13	0.21	0.17	0.14	1.40	0.87	0.58
VII. GERANIALES									
14. Burseraceae									
Canarium villosum F. Vill.	0.66	0.49	0.31	0.86	0.54	0.34	1.26	1.00	0.50
15. Meliaceae									
Xylocarpus granatum Koen. var.	0.47	0.36	0.13	0.67	0.37	0.23	1.39	0.97	0.61
16. Euphorbiaceae									
Excoecaria Agallocha L.	0.87	0.59	0.29	0.87	0.63	0.41	1.17	0.80	0.56
Glochidion littorale Bl.	1.28	0.90	0.36	1.21	1.04	0.72	1.84	1.52	0.92
VIII. SAPINDALES									
17. Anacardiaceae									
Anacardium occidentale L.	0.56	0.42	0.27	0.70	0.44	0.25	0.88	0.60	0.47
Buchanania arborea Bl.	0.63	0.41	0.29	0.61	0.41	0.27	1.17	0.97	0.34
Koordersiodendron pinnatum Merr.	0.70	0.52	0.29	0.83	0.64	0.41	1.69	1.17	0.74
Mangifera monandra Merr.	0.72	0.52	0.29	0.83	0.57	0.39	1.21	0.92	0.63
Semecarpus cuneiformis Blanco	0.52	0.29	0.25	0.56	0.43	0.29	1.12	0.79	0.54
18. Sapindaceae									
Guioa Perrottetii Bl.	0.45	0.38	0.32	0.66	0.43	0.25	2.00	1.48	0.96
Sapindus Saponaria L. var. *Turczaninowii* Vidal	0.41	0.25	0.14	0.50	0.33	0.19	1.60	1.20	0.68
19. Aceraceae									
Acer rubrum L.	0.64	0.49	0.27	0.61	0.49	0.32	1.24	0.84	0.50
IX. MALVALES									
20. Tiliaceae									
Columbia serratifolia DC.	0.57	0.43	0.30	0.57	0.45	0.37	1.72	1.34	1.04
Grewia multiflora Juss.	0.34	0.25	0.14	0.37	0.25	0.16	1.09	0.75	0.48
21. Malvaceae									
Thespesia populnea (L.) Soland. ex Corr.	0.32	0.25	0.14	0.28	0.25	0.21	1.45	1.09	0.36

TABLE 1 (*Concluded*)

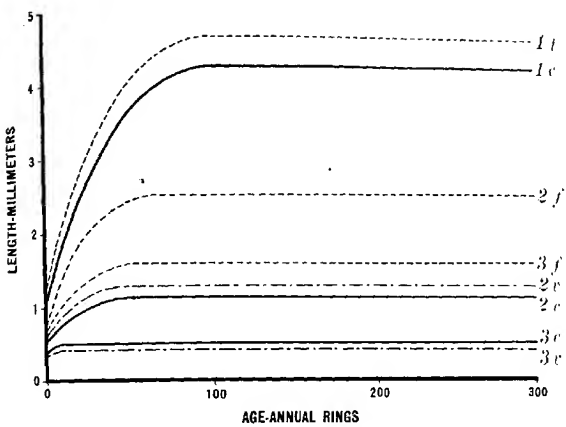
	Vessel-segments			Cambial Initials			Fiber l	
	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Ma...
22. Bombacaceae								
Bombycidendron Vidalianum Merr. and Rolfe	0.43	0.35	0.28	0.43	0.36	0.32	2.00	1.[illegible]
23. Sterculiaceae								
Heritiera littoralis Dryand.	0.37	0.31	0.25	0.36	0.30	0.27	1.88	1.4[illegible]
Kleinhovia hospita L.	0.50	0.33	0.19	0.48	0.36	0.27	1.24	0.8[illegible]
Pterospermum niveum Vid.	0.50	0.37	0.19	0.43	0.37	0.30	1.90	1.4[illegible]
Sterculia foetida L.	0.48	0.35	0.30	0.45	0.37	0.32	2.72	2.1[illegible]
Tarrietia sylvatica Merr.	0.34	0.27	0.18	0.34	0.28	0.21	1.96	1.5[illegible]
X. PARIETALES								
24. Guttiferae								
Calophyllum Blancoi Pl. and Tr.	0.99	0.61	0.36	0.90	0.59	0.41	1.35	0.90
Garcinia dulcis Kurz.	1.24	0.80	0.48	1.09	0.80	0.52	2.88	2.01
Garcinia sp. (probably *lateriflora* Bl.)	1.04	0.78	0.48	1.02	0.74	0.52	2.52	1.88
25. Dipterocarpaceae								
Anisoptera thurifera Bl.	0.66	0.48	0.36	0.72	0.54	0.41	2.12	1.68
Vatica Mangachapoi Blanco	0.79	0.58	0.36	0.81	0.61	0.41	1.63	1.15
XI. MYRTIFLORAE								
26. Lythraceae								
Lagerstroemia speciosa (L.) Pers.	0.43	0.30	0.18	0.50	0.33	0.21	1.52	1.08
27. Lecythidaceae								
Barringtonia racemosa (L.) Roxb.	0.97	0.68	0.39	0.90	0.72	0.50	3.84	2.51
28. Rhizophoraceae								
Bruguiera parviflora W. and A.	1.20	0.91	0.60	1.28	0.99	0.64	1.88	1.32
Rhizophora sp. (probably *Candelaria* DC.)	0.95	0.59	0.46	0.95	0.73	0.30	2.12	1.56
29. Nyssaceae								
Nyssa sylvatica Marsh.	1.72	1.25	0.88	1.27	0.83	0.54	2.52	1.76
XII. UMBELLIFLORAE								
30. Araliaceae								
Sheffiera odorata Merr. and Rolfe	1.00	0.82	0.56	0.97	0.84	0.66	0.76	0.52
B. METACHLAMYDEAE								
XIII. CONTORTAE								
31. Oleaceae								
Fraxinus americana L.	0.48	0.31	0.18	0.37	0.29	0.18	1.38	0.96
XIV. RUBIALES								
32. Rubiaceae								
Ixora philippinensis Merr.	1.13	0.62	0.50	1.17	0.76	0.43	1.78	1.18
Psychotria luzoniensis F. Vill.	0.95	0.67	0.37	1.08	0.70	0.45	1.53	1.12

VARIATIONS IN CROSS-SECTIONAL AREA AND VOLUME

The variations in the length of cambial initials are not neutralized by concomitant changes in the radial and tangential diameters of the cells. On the contrary, the cross-sectional area of the elongated meristematic

cells tends to be somewhat larger in old than in very young stems, and in most gymnosperms than in dicotyledons. In other words, the basic distinctions in *length* are paralleled by similar variations in width.

The tracheary elements of the secondary xylem tend to increase in volume during differentiation. In the case of the tracheids of Coniferae this increase is due primarily to "radial" expansion and secondarily to elongation. The tangential diameter of the developing tracheids remains nearly constant. In arborescent and fruticose dicotyledons, on the other



TEXT FIG. 3. Normal length-on-age curves for cambial initials and tracheary cells in (1) typical conifer, (2) dicotyledon having primitive vessels, and (3) dicotyl having highly specialized vessels. *c*, cambium; *t*, tracheids; *f*, fiber tracheids; *v*, vessel-segments.

hand, the volume of fiber tracheids tends to be much influenced by elongation, and that of the vessel-segments by "tangential" as well as by "radial" expansion. As indicated by Sanio (1872) for *Pinus sylvestris* L., by Hartig and Weber (1888) for *Fagus sylvatica* L., and by Prichard and Bailey (1916) for *Carya ovata* (Mill.) K. Koch, the cross-sectional area and volume of tracheary cells tend to be larger in the outer than in the innermost growth layers of the stem. In gymnosperms, the changes in the volume of the tracheids in succeeding annual rings are closely dependent upon variations in the length and volume of the cambial initials, whereas, in many of the more highly specialized dicotyledons, the fluctuations in volume of the fiber tracheids and vessel-segments in various parts of the stem are due largely to changes which occur during the differentiation of the tracheary elements. In the dicotyledons as a group, the shortening of the cambial

initials and fiber tracheids—which is closely correlated with the development and specialization of vessels—results in a reduction in volume of these elements, but the decrease in length of the vessel-segments frequently is more than compensated for by an increase in their cross-section. Thus, there is less contrast between the volume of the tracheids in gymnosperms and that of the vessel-segments in dicotyledons than there is between the size of the cambial initials in the two groups of plants.

Significance of Size Variations in Cambium and Xylem

These fundamental types of cell size variations, and concomitant fluctuations in form and structure, are significant in the investigation of a number of cytological, morphological, and physiological problems, as well as in the study of the identification and mechanical properties of timber, and will be discussed in greater detail in subsequent papers.

In view of the numerous factors or complexes of factors which affect the dimensions and volume of cells, it is not surprising that contradictory conclusions have been reached by different investigators who have attempted to generalize concerning cell size after limited induction. The data at hand indicate very clearly that the undifferentiated, actively dividing and growing cells of the lateral meristem or cambium may vary greatly in size in certain plants and remain relatively constant in others. Therefore, very different conclusions concerning the constancy of cell size or of cell number may be expected from intensive experimental investigations, depending upon the particular plant or portion of a plant which is selected for study. Similar discrepancies may be expected concerning body size and cell size. Depauperate plants (physiological dwarfs) frequently have smaller tracheary cells and cambial initials than individuals of normal stature, indicating a close correlation between cell size and body size. On the other hand, a large dicotyledon may be composed of much smaller cells than a small conifer or dicotyledon of similar age, suggesting that variations in cell size are independent of fluctuations in body size.

Sachs (1892, 1893, 1895) and Strasburger (1893) almost simultaneously called attention to the fact that undifferentiated, actively dividing and growing cells of plants, such as occur in embryonic and meristematic tissues, are relatively minute, and concluded that this is undoubtedly due to the fact that the working sphere of the nucleus is very restricted. Strasburger found that in terminal meristems the ratio between the average diameter of the nuclei and of the cells is as 0.003–0.16 mm: 0.005–0.24 mm., or 2 : 3, and Sachs pointed out that, although plants vary enormously in their linear dimensions (0.001 mm. to 100 m.), the size of their constituent cells is relatively constant (0.001 to 0.05 mm.). Winkler (1916) reaches similar conclusions. He states that in meristematic somatic tissues the cells are of nearly uniform size and contain the diploid number of chromosomes, whereas in non-meristematic somatic tissues, in which multinucleate protoplasts, nuclear

sions, and changes from the diploid to the tetraploid or polyploid condition are of frequent occurrence, many cells depart widely from the inherited, specific cell size of the plant. Therefore he suggests that there is a close correlation between cell size and chromosomal mass in both meristematic and non-meristematic somatic tissues.

Reconnaissance surveys of the higher plants indicate that the cambium should provide a favorable medium for testing the validity of these and similar generalizations concerning cell size, the working sphere of the nucleus, and the nucleo-cytoplasmic relation. Not only does the average size of the cambial initials fluctuate greatly in different groups of the Siphonogama, in different parts of a given individual, and in plants grown under different environmental conditions, but adjacent elements of the lateral meristem vary considerably in length, cross-sectional area, and volume. The cambial initials are of two distinct shapes and sizes: (1) numerous large, elongated cells, whose size variations have been described on preceding pages, and (2) scattered aggregations of small, more or less isodiametric elements which divide to form the horizontal sheets of radially disposed parenchyma, so-called medullary rays. The bulk of the divisions in both types of initials is periclinal, or parallel to tangents to the circumference of the stem or root. In other words, the large cells divide in a tangential, longitudinal plane which is a division plane of *maximal* area, whereas the ray initials form partition membranes that commonly are surfaces of *minimal* area. In gymnosperms and less highly differentiated dicotyledons, the cambium does not increase its periphery by radial, longitudinal divisions of the elongated initials and lateral enlargement of the products of such divisions. Instead, the cells elongate, sliding by one another, until they have attained a certain length. They then divide, by means of a pseudo-transverse partition, into two short halves which in turn elongate and divide.[8] Owing to the fact that the initials do not elongate and divide (transversely) in unison, there is usually a very considerable variability in the length and *pari passu* in the volume of adjacent fusiform elements. However, the volume of the more or less isodiametric ray initials is very much less than that of even the smallest fusiform initials, and is of the same general order of magnitude as that of the undifferentiated cells of the embryo or terminal meristem. Therefore, in any particular portion of the cambium of these plants it is possible not only to study cell division and the nucleo-cytoplasmic relation in adjacent fusiform initials of very different lengths and volumes, but to contrast them with similar phenomena in adjoining ray initials, which resemble the cells of the terminal meristem in size and shape. Furthermore, by proper experimental methods, the fusiform initials may be induced to divide into small isodiametric units of the general order of magnitude of the ray initials or embryonic cells, and subsequently to regenerate elongated elements of normal dimensions.

[8] During this process of elongation, between successive transverse divisions, the cells continue to divide in the tangential, longitudinal plane.

A number of interesting cytological problems suggest themselves in this connection. (1) Are the large, elongated initials multinucleate or hyperchromatic in conformity with the generalizations of Sachs, Strasburger, Winkler, and others? (2) Do the nuclei divide mitotically or amitotically? (3) What is the nature of cytokinesis in cells which are several hundred times as long as they are wide, and yet divide longitudinally? These and similar questions will be considered in the next paper of this series.

SUMMARY

1. Reconnaissance surveys of the higher plants reveal striking variations in the dimensions and volume of the cells of the cambium and secondary xylem.

2. Certain of the size variations are purely somatic, whereas others are germinal.

3. In many plants the dimensions and volume of tracheary cells are determined primarily by those of the cambial initials, whereas in others they are due largely to changes which occur during the differentiation of the xylem.

4. These fundamental types of size variations, and concomitant fluctuations in form and structure, are significant in the investigation of various cytological, morphological, and physiological problems.

5. The cambium appears to be an unusually favorable medium for the study of problems relating to cell size and body size, the working sphere of the nucleus, the nucleo-cytoplasmic relation, and phenomena of cytokinesis in somatic tissues.

In conclusion, the writer wishes to express his indebtedness to Doctor E. D. Merrill, Director of the Philippine Bureau of Science, for his kindness in sending carefully preserved and identified specimens of a number of tropical plants.

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AN APPARATUS FOR DETERMINING SMALL AMOUNTS OF CARBON DIOXIDE

R. C. WRIGHT

Certain investigations carried on by the Bureau of Markets in connection with the storage of fruits and vegetables require a simple and rapid method of determining small quantities of carbon dioxide in the air of both common and cold storage plants. The Orsat apparatus has been used to some extent in this work, but is open to objection because it will not measure small enough quantities, and the apparatus is somewhat heavy to carry

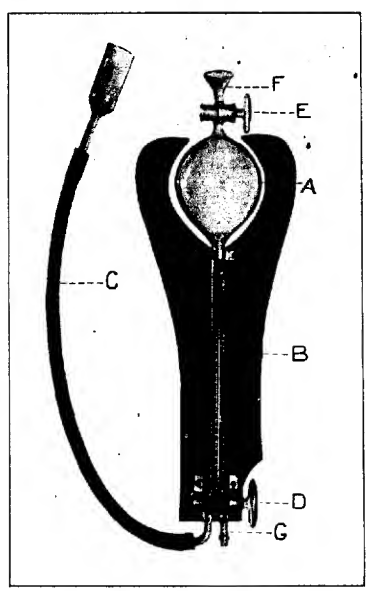

FIG. 1. See text for explanation.

about. Titration methods are, of course, the most accurate, but the necessary equipment, which is not easily portable, makes these methods practical only in what might be termed stationary experiments and where extreme accuracy is necessary.

The volumetric apparatus which has been developed by the writer has the advantages of being light, easily portable, measuring only 14 × 4 inches, and so simple in construction that it can be used by an unskilled operator. The apparatus is made entirely of glass and mounted on wood, and makes determinations ranging from 0.1 to 3.0 per cent. The calibrations, however, are sufficiently far apart so that readings by interpolation may be made to 0.05 percent. The apparatus can readily be used in the close, cramped quarters and poor light often found within storage rooms. Each determination takes from three to five minutes.

The carbon dioxide apparatus described herewith (see figure 1) consists of a bulb *A* and stem *B* of about 150 cc. capacity, a stopcock *E*, a balance tube *C*, a two-way stopcock *D*, and a funnel *F*. The apparatus is filled with air to be analyzed, and sodium hydroxide is introduced to absorb the carbon dioxide which is replaced by water entering from the balance tube *C*. The height of the column of water in the tube *B* gives directly in percentage the amount of carbon dioxide removed from the sample of air.

Following is a description of the method of operating the carbon dioxide apparatus. Wet the inside of bulb and stem *B*, then drain one minute. Fill the balance tube *C* with water. The water should rise in the balance tube just to fill the bore in stopcock *D*. Be sure no air bubbles are left inside the rubber tubing. Turn the stopcock *D* to make connection with the outlet *G*. Open the stopcock *E*, and by means of a bulb attached at *G*, pump into the apparatus sufficient air to get a representative sample, or place the mouth over *G* and draw through the apparatus sufficient air to get a good representative sample within. Turn *D* to connect with *C*. Lower the balance tube *C* till the level of water within is slightly below the bottom of tube *B*. Partially fill funnel *F* with a saturated solution of sodium hydroxide. Allow this to enter the apparatus slowly. Close *E* and raise the balance tube to allow two or three cubic centimeters of water to enter tube *B* along with the sodium hydroxide, then close *D* and tip the apparatus to allow the liquid to run into bulb *A*. Shake gently to allow the liquid to splash about in bulb *A* to facilitate absorption of carbon dioxide. Turn the apparatus upright. Open the stopcock *D* to connect with the balance tube *C*. Raise and lower the balance tube *C* as far as possible five or six times to force the rapid diffusion of sodium hydroxide, thus making the liquid in *C* of uniform density throughout. Allow liquid to drain down from the side of the apparatus for one minute, then hold the balance tube so that the top of the column of liquid within is on a level with that in tube *B*—thus correcting for atmospheric pressure. Read the height of liquid in tube *B*. (Because of the unequal capillarity due to the difference in diameters of tube *B* and the top of leveling tube *C*, when making a reading hold *C* so that the top edge of the meniscus is on a level with the bottom of the meniscus in *B*.) The reading gives directly in percentage the amount of carbon dioxide originally present. Rinse out after each determination.

When it is desired to analyze a sample of air from a container, such as a barrel or box, attach a bulb at *G* as usual, then connect the intake end of the bulb with a tube through which air from the container may be pumped, or attach a rubber tube at *G* through which air may be drawn from the container by placing the mouth over the funnel *F*, taking precautions to wash off all sodium hydroxide from about the sides of the funnel.

When operating the apparatus gloves should be worn and it should not be held close to the body, as the heat will expand the air within and true results will not be obtained.

BUREAU OF MARKETS,
U. S. DEPARTMENT OF AGRICULTURE

THE SECRETION OF INVERTASE BY PLANT ROOTS

LEWIS KNUDSON

In an earlier paper (Knudson, 3) on the utilization of certain carbohydrates by green plants, the observation was repeatedly made that reducing sugars appeared in culture solutions containing sucrose. In discussing the possibility of invertase secretion the following statements were made:

"It has not yet been definitely proved that the inversion of saccharose is due to invertase secreted into the culture solution. It is possible that the saccharose is inverted in the roots and the reducing sugars are secreted, but this is less probable. It is possible also that the enzyme may be released as a result of the death of root hairs or other cells of the root and that it is not secreted from living cells."

A few observations have been made by other investigators on this subject of enzyme secretion, but the observations have been only incidental to other investigations and the few reports are conflicting. It seemed desirable therefore to investigate thoroughly the possibility of enzyme secretion and particularly that of invertase, since it is this enzyme that is most likely to be found in the roots of plants. Accordingly the investigation here reported was undertaken. Not all the experiments performed are reported, but those omitted are in agreement with the results here given.

METHODS

The methods employed are essentially the same as those used by Knudson and Smith (4) in their experiments on the secretion of amylase. The plants were grown in water cultures under sterile conditions, that is, with the root system in a nutrient medium free of microorganisms and the seed removed from contact with the culture solutions. The type of culture is shown in figure 1. The details of manipulation are sufficiently described by Knudson and Smith (4) and need no repetition here.

Pfeffer's nutrient solution was used, with the substitution, however, of dibasic potassium phosphate for the monobasic potassium phosphate. The solution was prepared according to the following formula: $Ca(NO_3)_2$ 4 grams, K_2HPO_4 1 gram, KNO_3 1 gram, $MgSO_4 \cdot 7H_2O$ 1 gram, KCl 0.5 gram, $FeCl_3$ 50 milligrams, distilled water 6 liters. To this solution was added, when desired, sucrose.

In the use of Pfeffer's solution, according to the formula given, it is essential that precautions be taken to prevent acidification of the culture solution. When the nutrient solution is sterilized in an autoclave for a period of 30 minutes or more, there may result an acid solution. This appears to be due to the reaction between calcium nitrate and dibasic potassium phosphate, whereby there is produced some tri-calcium phosphate

with the liberation of some nitric acid. If the sucrose is present, th... re, in the nutrient solution, a considerable portion may be inverted. The period of sterilization seems to be a factor in this acidification, for in ... of the preliminary experiments no such inversion occurred when th... ire nutrient solution was sterilized at one time.

Throughout the experiments here reported the following meth... was adopted. The nutrient solution was made up in two portions. Por... n .1

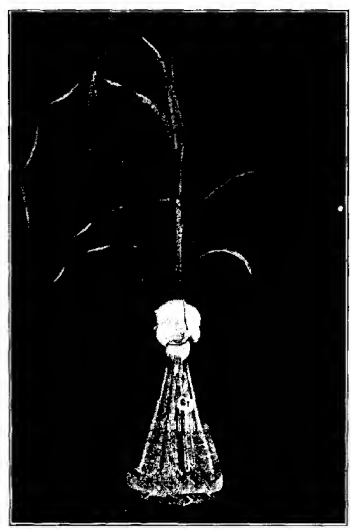

FIG. 1. See text for explanation.

includes all the salts except calcium nitrate. Portion *B* includes calcium nitrate only. To solution *A*, which is slightly alkaline, the sucrose was added. The solutions were originally made up double strength so that equal quantities of *A* and *B* would give the desired concentration of the sucrose and salts necessary for the nutrient solution. The method of procedure is as follows: When 1100 cc. of the culture solution is desired, 550 cc. of each solution (*A* and *B*) is accurately measured out. Solution *A* is placed in the culture flask and this is provided with a cotton stopper with a central tube. Through this tube is inserted the stem of a 9-centimeter funnel, and the funnel and neck of the flask are then covered with cotton to prevent any contamination after sterilization. Solution *B* is placed in a liter flask stoppered with cotton and the stopper and neck are also enclosed

with cotton. The two flasks are then sterilized in an autoclave for 30 minutes at a pressure of 15 pounds. When the solutions are cool, solution *B* is poured into the culture flask containing solution *A*. This transference of solution *B* to *A* takes place under conditions to minimize as much as possible the possibility of contamination. The funnel is then replaced by a cotton stopper and the cotton stopper and neck of the flask are covered again with cotton to prevent organisms and spores from lodging in the cotton stopper, circumstances which might cause contamination when the seedling is transferred to the culture flask. The flasks are permitted to stand several days before the seedlings are transferred, and at the time of transplanting any that show contamination are rejected.

Hydrogen-ion determinations were made by the indicator method, using mixtures of monobasic potassium phosphate and dibasic sodium phosphate prepared according to the methods of Sorensen (Prideaux, 7). These determinations were made at the outset of the experiment and also at its conclusion, and in some experiments mentioned subsequently the reaction of the culture solution was followed by adding to the culture solution the indicator, neutral red. The hydrogen-ion concentration is expressed as the logarithm (the base being 10) of the normality with respect to the hydrogen ions. The minus sign is understood; for example, P[H]7 refers to a hydrogen-ion concentration of 10^{-7} normal.

Sugar determinations in experiment 1 were made by Kendall's method (2), and the reducing sugar is expressed as invert sugar. In all the other experiments the volumetric method of Cole (1) was used. This method proved to be a rapid and accurate method for the purpose. The reducing sugars are expressed as glucose. In the use of both methods the solutions were standardized against prepared sugar solutions, the sugars used being of a very high degree of purity.

EXPERIMENTS

Experiment 1. In this experiment Canada field pea (*Pisum arvense* L.) was used. The culture vessels were pyrex flasks of one-liter capacity and the quantity of the nutrient solution was 1050 cc. Sterilization of the solutions was effected by autoclaving at 15 pounds pressure for 30 minutes. Seeds were sterilized by the use of calcium hypochlorite for one hour. The plants were grown in a greenhouse at an average temperature of 70° C.

At the conclusion of the experiment the culture solutions were tested for sterility by plating 1 cc. of each solution on an agar medium of Pfeffer's solution plus 1 percent sucrose. Only those cultures that proved to be sterile were analyzed. The results follow in table 1 and a typical culture is shown in figure 1.

There is in each of the culture solutions containing sucrose an appreciable gain in reducing sugars, but the gain is relatively slight as compared to the total amount of sucrose present. If the enzyme invertase is secreted, why

TABLE 1. *Canada field pea. Duration, Nov. 2 to Dec. 13, 1916: 42 days.*

Culture Solution	Culture Number	Water Transpired (Cubic Centimeters)	Dry Weight			Total Sugar in Culture Solution at End of Experiment, Calculated as Invert Sugar (Grams)	Sugar Absorbed by Plant, Calculated as Invert Sugar (Grams)	Reducing Sugar in Culture Solution at End of Experiment Calculated as Invert Sugar (Grams)	
			Roots (Grams)	Tops (Grams)	Total (Grams)				
Pfeffer's + ½ per cent sucrose	1	210	0.130	0.375	0.505	4.584	0.216	0.541	0.233
" + ½ per cent "	2	230	0.170	0.368	0.538	4.544	0.256	0.600	0.292
" + ½ per cent "	3	170	0.082	0.215	0.297	4.652	0.148	0.497	0.189
" + ½ per cent "	4	120	0.140	0.140	0.280	4.572	0.228	0.491	0.183
" + ½ per cent "	5	150	0.100	0.280	0.380				

is there not a greater production of reducing sugars? The maximum increase in reducing sugar is only one fifteenth of the sucrose present.

Circumstances prevented at this time any incubation experiment with the culture solutions to determine whether or not there would result an increase in reducing sugars which might be taken as evidence of the presence of invertase.

Experiment 2. The culture methods and conditions were essentially like those of the preceding experiment. The nutrient solution was slightly modified by the substitution of ferrous chloride for ferric chloride, and the sucrose used was Merck's highest purity. The nutrient solution at the outset had a hydrogen-ion concentration of P[H] 6.80.

Two plants were used in the experiment: corn, variety Weber's Dent, and Canada field pea. Unfortunately most of the cultures of Canada field pea became contaminated, and data were obtained from only one sucrose culture.

An examination of table 2 reveals the fact that as usual a better growth is obtained with sugar than without. An exception is culture number 6, which was maintained in diffused light in the laboratory for ten days preceding the conclusion of the experiment.

In cultures 6 to 10 inclusive there was noted an increase in reducing sugars, but none was found in cultures 11 to 15 inclusive. The amount of reducing sugar in the sucrose cultures, while appreciable, is again relatively small compared to the total sugar present. In culture number 8 the unusually large amount of reducing sugar was undoubtedly due in part to contamination by a species of Penicillium which made its appearance during the last week of growth. The average hydrogen-ion concentration was at the conclusion of the experiment P[H] 7.35.

In order to determine whether or not the enzyme invertase is present in the culture solution, 500 cc. portions of the solutions were incubated for 14 days at a temperature of 32° C. As an antiseptic agent, 2 percent of

TABLE 2. *Corn. Duration, Dec. 31, 1918 to Feb. [illegible], 1919.*

Culture Solution	Culture Number	Water Transpired (Cubic Centimeters)	Green Weight (Grams)	Dry Weight: Roots (Grams)	Dry Weight: Tops (Grams)	Dry Weight: Total (Grams)	[illegible]	[illegible]	[illegible]	[illegible]
Pfeffer's + sucrose . . .	6	300	21.5	0.220	0.970	1.19	3.761	0.984	0.555	
" + " . . .	7	326	27.0	0.410	1.420	1.83	1.311	0.430	0.431	
" + " . . .	8	450	29.0	0.540	1.620	2.160	3.057	1.006	0.956	
" + " . . .	9	340	29.0	0.290	1.30	1.590	1.217	0.535	0.305	
" + " . . .	10	270	23.0	0.270	1.250	1.520	4.895	0.158	0.328	
" + " . . .		Control solution no plant					1.755		11.0[illegible]	
Pfeffer's	11	300	13.2	0.120	0.610	0.730				
"	12	310	16.5	0.230	1.050	1.280				No reducing sugar
"	13	190	11.5	0.220	0.650	0.870				
"	14	168	10.5	0.130	0.520	0.650				
"	15	300	20.0	0.190	1.100	1.290				

[1] Kept in laboratory in diffused light for 10 days before analysis.
[2] Contaminated.

toluene was used. At the end of the 14 days, analyses were again made for reducing sugars. No increase was shown in any case after incubation except in number 3. In this case the amount of reducing sugar had nearly doubled, due undoubtedly to the enzyme invertase derived from the Penicillium contamination.

In the Canada field pea cultures only one of the sucrose cultures remained uncontaminated. The duration of growth in this case was 50 days, the green weight 14.95 grams, and the amount of reducing sugar present 0.448 gram. The total sugar present calculated as sucrose was 3.711 grams, and the amount of sugar as sucrose used was 1.042 grams. The non-sucrose cultures did not show any reducing sugars in the culture medium. As in the experiment with corn, 500 cc. of the culture solution was incubated for 14 days at a temperature of 32° C. No increase in reducing sugar was found at the end of that period.

Experiment 3. A white dent variety of corn was used and the plants were grown as before in the greenhouse. The duration of the experiment was from June 27 to July 29, a period of 32 days. The concentration of sucrose was ½ percent. In this experiment the hydrogen-ion concentration of the culture solution was again accurately determined by the indicator method, using anhydrous KH_2PO_4 and Na_2HPO_4 according to Soerensen and using neutral red as the indicator. In addition to determining the hydrogen-ion concentration, several cultures were provided, to each of which was added 1 cc. of a ½ percent solution of neutral red, the purpose being to follow the reaction during plant growth. This was possible for only about ten days, for the plant by the tenth day had absorbed all the indicator. From the outset the solution became increasingly alkaline, so that it was only at the outset that an acid reaction prevailed and then the hydrogen-ion concentration was only $10^{-6.7}$ normal.

The results of experiment 3 are similar to those of the preceding experiments. There is the usual increase in reducing sugars; the reaction of the solution at the outset was very slightly acid (P[H] 6.7), and at the conclusion slightly alkaline (P[H] 7.25 to 7.3)

Incubation experiments were made as in the previous experiments.

TABLE 3. *Corn. Duration of experiment, 32 days.*

Culture Number	Sugar as Sucrose at End (Grams)	Sucrose Used by Plant (Grams)	Reducing Sugar at End (Grams)	Gain in Reducing Sugar (Grams)	P[H] at conclusion of Experiment
16.....	4.891	0.940	0.476	0.376	7.3
17.....	4.550	1.181	0.400	0.300	7.30
18.....	4.912	0.819	0.375	0.275	7.25
19.....	4.912	0.819	0.571	0.471	7.25
Control, no plant.....	5.731		0.100		6.70

The duration of the incubation experiment was 14 days. Toluene 2 percent was added as the antiseptic agent, and the temperature of incubation was 35° C. No increase in reducing sugar over that found in the culture solution was noted after the period of incubation.

Experiment 4. This experiment was like the preceding. Canada field pea was used and the results appear in table 4.

TABLE 4. *Canada field pea. Duration, Sept. 4 to Sept. 22: 18 days.*

Culture Solution	Culture Number	Dry Weight			Reducing Sugar Present	Gain in Reducing Sugars
		Roots (Grams)	Tops (Grams)	Total (Grams)		
Pfeffer's solution + sucrose.....	1	0.221	0.254	0.475	839	0.333
" " + "	2	0.186	0.322	0.508	877	0.371
" " + "	3	0.223	0.277	0.500	820	0.314
" " + "	4	0.200	0.260	0.460	876	0.370
" " + "						
no plant					506	
Pfeffer's solution.....	1	0.113	0.216	0.339	No reducing	
" "	2	0.127	0.309	0.436	sugars in these	
" "	3	0.083	0.169	0.252	solutions	
" "	4	0.099	0.169	0.268		

Experiment 5. Culture in distilled water. In order to determine whether or not the character of the nutrient solution had any special significance with respect to the increase in reducing sugars, an experiment was made using in place of Pfeffer's solution merely distilled water to which was added 1 percent sucrose. Canada field pea was used and the duration of the experiment was 18 days. The conditions and methods of the experiment were similar to those of the preceding experiments.

Only one culture remained uncontaminated. The green weight of the plant was 1.35 grams, the reducing sugar in the culture solution (1000 cc.) at the conclusion of the experiment was 293 milligrams, while in the control there was only 138 milligrams; there was an increase therefore of 155 milligrams in the culture solution.

An incubation experiment was also made. 500 cc. samples were taken from the culture solution and from the control solution, and toluene was added. The solutions were incubated at 35° C. and then again analyzed for reducing sugars. The increase after nine days was but 5 milligrams.

Nutrient solution minus iron salts. Rice and Osugi (8) in working on the inverting power of various soils have presented evidence that inversion of sucrose may be affected by various colloids and suggest that the inversion may be due to adsorbed acids. In the Pfeffer's solution after sterilization there is precipitated ferric hydrate, and this precipitate is increased after a few days' growth of the plant. In order to determine whether or not the ferric hydrate might be responsible for the increase in reducing sugar, an experiment was performed in which iron was omitted from the culture solution. The methods were the same as for the previous experiments. Sucrose was supplied at a concentration of 0.5 percent. Corn was again used and the plants were grown for 18 days in the greenhouse. The dry weights of tops and roots were 0.725 grams and 0.200 grams respectively. Analyses showed 500 milligrams of reducing sugars, while the control solution had only 305 milligrams. The increase was therefore 95 milligrams.

Discussion

What is the cause of the increase in reducing sugar in the culture medium? Is the enzyme invertase excreted? The evidence is contrary to this idea. In no case was there obtained any increase in reducing sugar after incubation. It is possible, of course, that the enzyme invertase is excreted from the root in such small amounts that the reaction effected is very slight. It might be suggested, furthermore, that the culture solution is unfavorable to the invertase and that the latter is soon destroyed. It was noted, however, that whenever the culture solution became contaminated with a yeast or a fungus, there was a marked increase in reducing sugars, and that this increase continued after incubation. The incubation experiment for culture number 8 of experiment 2 yielded data in support of this statement. In accordance with the view of Rice and Osugi (8) it might be expected that the mucilaginous matter of the root and surrounding the root-cap cells as well as the cell walls might adsorb basic ions, the process resulting in a preponderance of hydrogen ions which might cause inversion of sucrose. But since the culture solution becomes increasingly alkaline in reaction with the advent of time, and since this alkalinity is due to the absorption of anions by the roots, it is reasonable to conclude that the zone about the roots is constantly of greater alkalinity than the "outer" regions of the culture solutions. In other words, the gradient of concentration of hydroxyl ions falls with increasing distance from the roots.

There is still another alternative. The cells of the root-cap are sloughed off, and it might be suggested that the root cells in dying yield reducing sugar to the culture solution. But, as stated in another paper (Knudson, 5),

the writer has found that the root-cap cells that accumulate at the bottom of the culture flasks are not dead but apparently remain alive for a considerable period. Examination of the sloughed off root-cap cells at the conclusion of the experiments revealed that they were alive and in good condition. Furthermore, the total weight of such cells would not be over 20 milligrams.

It seems to the writer that there is only one explanation to account for the accumulation of reducing sugars, and that is excretion of reducing sugars by the roots.

In accordance with this view, the procedure might be as follows: Sucrose is absorbed by the roots and inverted in the root cells by the enzyme invertase. Some of the sugar is used in growth, but there is a superabundance of reducing sugars and they accumulate in the root cells. At the outset there are practically no reducing sugars in the sucrose solutions. The concentration gradient between the reducing sugars in the cells and those outside is steep, and consequently some of the reducing sugars diffuse outward. With the progress of time the difference in concentration of reducing sugars becomes less, but probably it is considerable at all times, since at the conclusion of the experiment the concentration of the sucrose in the culture is much greater than that of the reducing sugars; and since there is a constant inward diffusion of sucrose, there results a constant production of reducing sugars in the plant cells.

In support of the view that the reducing sugars are excreted, the following experiment may be cited. Three corn plants which had grown for 30 days in Pfeffer's solution, each plant having a fresh weight of approximately 18 grams, were removed from the culture vessels and the roots washed in tap water. At 5 p.m. the plants were transferred to culture vessels so that their roots alone were bathed in a four percent solution of sucrose (Merck's highest purity). Three culture vessels were used and 400 cc. of the solution. The roots were kept in this solution for 16 hours. The plants were then removed and rinsed seven times in tap water, and then the plants were transferred to culture vessels this time containing distilled water. The plant roots remained in distilled water 7 hours. The total volume of distilled water was then reduced by evaporation to 100 cc. This was analyzed for reducing sugar and the determinations gave 14.5 milligrams of reducing sugar.

In another experiment plants were used which had been growing in a nutrient solution plus sucrose, and treated in the same way as in the preceding experiment. There were leached from the roots of four plants 75 milligrams of reducing sugar and 150 milligrams of sucrose.

The secretion of sugars by the roots of plants may seem at the outset to be a rather startling idea, yet theoretically there is no reason why this should not occur. Wächter (9) reported considerable excretion of sugars by slices of beets and onions when immersed in distilled water or in salt

...ations, and recently much evidence has been presented showing the le... hing of electrolytes from the roots of plants (see Merrill 6 for a review of literature).

Summary and Conclusions

1. Evidence is presented to show that Canada field pea (*Pisum sativum* L.) and corn (*Zea mays* L.) grown in the presence of sucrose cause an increase in reducing sugars in the culture solution.

2. The reaction of the culture solution is such as to be without influence on the sucrose.

3. Incubation experiments yielded negative results with respect to the presence of invertase.

4. The idea is held that the increase in reducing sugars is due to excretion of these from the roots.

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DAILY RHYTHMS OF ELONGATION AND CELL DIVISION IN CERTAIN ROOTS[1]

RAY C. FRIESNER

INTRODUCTION

The subject of periodicity of growth activities in plants is by no means a new one; in fact, it is one of the oldest. But a careful review of the available literature shows that there are still certain phases of the work which have not yet been thoroughly investigated. Two of these are embodied in the present paper, *viz.*, rhythms of elongation and rhythms of cell division in roots under constant environmental conditions.

HISTORICAL

Elongation

Aerial parts. Sachs (29,30) gives an historical account of the older literature up to his time. No attempt will be made to reproduce it here except to point out that the work before his time was all done on large plants which grew rapidly and which in most cases had to be observed in the open where external factors could not be controlled. Hence, the work was only of the grossest nature and led to no definite general conclusions. In 1872 Sachs (29) published the results of his study of the elongation of the stem in various plants, including *Dahlia variabilis*, Fritillaria, Polemonium, etc. In general he found that plants exposed to the alternation of darkness and light exhibited a single daily wave of elongation in which the maximum occurred shortly after sunrise, and the minimum shortly after sunset. This he formulated into his so-called "universal law." He further found that this daily periodicity is entirely absent from plants grown continually in the dark.

In 1873 Prantl (27) found, in studying the rate of growth in leaves, that curves for increase in width are very similar to those for increase in length, and that under normal conditions the maximum is reached in the morning from 6 to 9 and the minimum in the evening from 6 to 9. He found, further, that by changing the hours of illumination and darkness he could shift the times of maxima and minima at will, since for each change in the time of illumination and darkness there was a corresponding change in the times of maxima and minima. These results show clearly that the daily periodicity here is an induced one. In continuous darkness this periodicity was absent. In 1878 Stebler (33) published the results of similar observations on the

[1] Papers from the Department of Botany of the University of Michigan, no. 180.

growth of leaves of various species among which were *Scilla sibirica*, *Pulmonaria officinale*, *Allium Cepa*, Cucurbita, Melampsora, etc. His results seem to show that the time of maximum growth coincides with the time of maximum light intensity and that of the minimum growth with the time of minimum light intensity. Here, also, a single daily wave of elongation and its use in width was found, though its precise relation to the time of environmental changes was somewhat different.

In 1879 Baranetzky (3) published the results of his investigations on a number of species including *Gesneria tubiflora*, *G. cardinalis*, *Helianthus tuberosus* (plants from tubers), *H. annuus*, *Brassica rapa*, etc. In brief, he found that plants which exhibit a regular daily periodicity when exposed to the alternation of darkness and light gradually lose this periodicity when placed in continuous darkness. The time required for complete loss varies from two to three days in the case of *Gesneria tubiflora* to 14 days in that of *Helianthus tuberosus*. Further, the intensity of the rhythms decreases from day to day. Plants grown from the beginning in darkness exhibited no periodicity except in the case of the shoots of *Brassica rapa*, some of which showed a very clear and regular rhythm, others a poorly defined one, and still others showed none at all. He regards this as due to heredity. It could hardly be considered such according to the commonly accepted use of the word heredity. A better term would be the "persistence of the habit" in the tuber, and its subsequent transference to the shoot.

In 1892 Godlewski (10) published the results of his researches on the growth of epicotyls of *Phaseolus multiflorus*. In the experiments carried out in June 1888, he found that plants growing under normal conditions exhibited a single daily wave of elongation, the maximum coming in the afternoon and the minimum near midnight. The following year plants grown from seeds of the same collecting showed the waves to come somewhat later, the maximum at evening and the minimum in the morning. Further experiments with seeds of a different lot gave two daily waves. Plants exposed to uniform conditions showed a very considerable variation. In some no marked rhythms were found, and in others rather irregular and unsteady ones were found.

Underground parts. The earliest work on underground parts was that of Strehl (36) in 1874, on the radicle of *Lupinus albus* L. The conditions of his experiments were, however, far from normal, inasmuch as the seedlings were grown with their roots in water and kept near a west window where they were exposed to moderately strong light. In plants thus subjected to the alternation of day and night he found in most cases a single daily wave of elongation with maximum coming near midnight and minimum near noon. In a few cases two waves were found.

In 1891 MacMillan (23) reported the results of his experiments upon the potato tuber. He found that tubers growing in continuous darkness exhibited rhythmic pulsations in their growth, showing two, three, and four

maxima and minima in the 24-hour period. He further found that the rhythms of the tuber were related to the periodicity of the aerial parts, but he thought it also probable that the tuber exhibited a rhythm of its own which was more or less obscured by the induced periodicity of the aerial parts. In 1901 Miss Gardner (9) reported the results of experiments on the growth of roots of *Pisum sativum* and *Vicia faba*. She found that roots exposed to the alternation of day and night elongated much more rapidly during the day than during the night. But the conditions of the experiment were far from normal, *viz.*, seedlings were placed in moist sawdust in wooden boxes with one glass face, and were made to grow in a horizontal direction.

A more recent work on elongation of underground parts is that of Kellicott (14) in 1904. In general, he found that curves for elongation of roots grown from bulbs of *Allium Cepa* exhibited three waves of elongation in the 24-hour period. Curves for different individuals were quite similar, though differing somewhat in the precise time of their maxima and minima. In general, the maxima came in the early morning and late afternoon and the minima came near noon and midnight. This work was done in the absence of any changes of environment, and hence is the first work definitely noting a regular rhythm not induced by external changes. A brief summary of the above account of investigations on elongation should note that (1) regular daily periodicity exists in the presence of regular daily changes in the environment; (2) this periodicity is gradually lost when the plants are exposed to constant conditions, though irregular and unsteady variations of the type called "autonomic" are reported; (3) the work of Kellicott on the root of Allium is the first to note any regularity in elongation of roots grown under constant conditions.

Cell Division

Lower plants. A great many statements are to be found in the older literature in regard to the time of day of nuclear and cell division among the Thallophytes. Thus Braun (4) notes that cell division in the formation of the gonidia of *Draparnaldia mutabilis* occurs between 6 A.M. and 11 A.M.; of *Stigeoclonium protensum*, between 6 A.M. and 10 A.M.; of *Cladophora tuberculata*, 8 A.M. to 2 P.M.; cell division in Spirogyra is most rapid during the night. Thuret (37) notes that the zoospores of Vaucheria are always liberated at about 8 A.M.; those of *Cutleria multifida* at daybreak; while those of *Enteromorpha clathrata* escape during the afternoon. Famintzin (8) corroborates Braun's statement in regard to Spirogyra. Strasburger (35) notes that cell division in Spirogyra is most rapid at 10–12 P.M., but may be delayed until the following morning if the plants are placed at a temperature of 0° to 5° C. during the night. De Wildeman (39), on the other hand, was unable to note any sensible difference, between day and night, in the rate of division in the cells of Spirogyra. His work was done

during the winter months from material collected outside. Klebs (11) reports Zygnema as dividing most frequently between 9 P.M. and midnight. Numerous other examples from the older literature are cited by Karsten (12) which will not be reproduced here. Karsten (13) in the most recent paper has shown that the desmids: *Cosmarium Botrytis*, *Cosmarium moniliferum*, and *Mesotaenium Endlicherianum*, when grown under normal conditions of illumination, exhibit a regular daily periodicity in the rate of nuclear and cell division. Cosmarium exhibits *three* waves. The primary maximum (about 50 percent of all cells) occurs at 1 A.M., with secondary maxima at 5 and 11 A.M. The primary minimum (about 5 percent of all cells) occurs at 1–3 P.M., with secondary minima at 3 and 7 A.M. Similarly, Closterium and Mesotaenium exhibit regular waves in the percentage of cells undergoing division, differing only in detail from the condition above noted for Cosmarium. It should be borne in mind that all of the above cited cases are reported from experiments carried on under normal conditions of light and darkness.

Aerial parts of higher plants. The only published reports on periodicity of cell division in aerial parts known to the writer are those of Karsten (12 and 13). He used as material the apical meristem of seedlings of *Pisum sativum*, *Zea Mays*, and *Pinus austriaca*. Seedlings of Pisum grown in continuous darkness showed a very marked increase in the number of cells undergoing division between 9:30 P.M. and 2 A.M., with a minimum falling at 6 A.M., while the remainder of the day was occupied with smaller fluctuations. Similarly, the curve for *Zea Mays* grown in continuous darkness shows numerous minor oscillations during the day, with a very marked rising during the night until the crest is reached at about 4 A.M., from which time it falls back again to the day position. *This rhythm is independent of changes in illumination and temperature.* The effect of alternation of darkness and light was then studied. When plants were lighted during the day and darkened during the night, much the same sort of curve was obtained as when in continuous darkness. When the times of illumination and darkness were reversed, two waves appeared with maxima at 6 A.M. and 6 P.M. and minima at 4 P.M. and 10 P.M. When the plants were continually lighted the waves were much shorter and more numerous. Seedlings of *Pinus austriaca*, when grown under normal conditions, showed maxima at 4 A.M. and 4 P.M. and minima at 12 M. and 6 P.M.

Underground parts of higher plants. The earliest work of this sort done on roots is that of Lewis (21). In the preliminary notice of this work it is shown that roots from bulbs of *Allium Cepa*, when grown in water and under normal conditions of illumination, *i.e.*, regularly alternating day and night, show two waves in their rate of cell division. The maxima come at midnight and noon, and the minima at 4 A.M. and 4 P.M. When yellow light was used the maxima appeared as before, but with the minima at 8 A.M. and 8 P.M. In blue light the maxima occurred at 4 A.M. and

noon, with the minima at 8 A.M. and 4 P.M. Finally, in continual dark[illegible] the maxima came at 4 P.M. and 8 A.M. with the minima at midnight [illegible] noon. Two waves were found in all these curves. The work of Kell[illegible] (14) also shows two waves in the curves for cell division in roots of *Allium Cepa* grown from bulbs and in moist sawdust. The maxima came at 11 P.M. and 1 P.M. and the minima at 3 P.M. and 7 A.M. It should be noted that in his curve I no figures are given for 5 A.M., and that in his curve II the curve rises from the "normal" 11 P.M. maximum to a much higher one at 5 A.M. This point will be referred to again in connection with my own results. It should also be noted that a total difference of 13° C. appears between the highest and lowest temperatures, though there is apparently no direct relation to be noted in the curves between these temperature changes and changes in rate of cell division. Roots of *Podophyllum peltatum* also showed rhythms in their curve of cell division, though they were more numerous than in Allium.

Karsten (12) studied cell-division in the root tips of *Vicia faba* and *Zea Mays*. The curve for *Vicia faba* showed marked maxima at 9 A.M. and 9 P.M. with minima at 4 P.M. and 7 A.M., and a few minor variations. The curve for *Zea Mays* showed smaller oscillations throughout the entire 24-hour period, though the curve is higher from 5 A.M. to 6 P.M. and lower from 6 P.M. to 5 A.M., the highest point being reached at 7 A.M. and the lowest at 9 P.M. These experiments were conducted in continuous darkness.

Miscellaneous

It is of interest and indirect bearing on the present paper to mention a few other cases in which either rhythm or a daily periodicity is found. Pfeffer (26) found nearly the same results in regard to sleep movements of leaves, *viz.*, plants subjected either to constant illumination or to constant darkness lose their regular daily periodicity. In some cases autonomic waves are found under uniform conditions, and in others they are entirely absent. When present they show considerable variation both in different individuals and in different leaves of the same plant. Baranetzky (2) and Detmer (7) have shown that there is a single wave in the daily curve for root pressure. The maximum, while varying somewhat in different individuals, comes some time in the afternoon and the minimum about 12 hours later. In a recent paper, Romell (28) reports the same results from plants continually lighted: "Die Dauerlichtpflanzen, ohne Ausnahme, eine sehr ausgeprägte Tagesperiodicität in der Blütungskurve besässen." Humphreys (11) calls attention to the presence of two maxima and two minima in daily atmospheric pressure, and in electrical potential. Similarly, Dechevrens (6) reports, from observations in Jersey, the presence of a diurnal rhythm in electrical potential of the atmosphere. Kraus (15, 16) and Millardet (24) have shown that the daily periodicity of tissue tension is gradually lost when the plants are exposed to uniform conditions. Finally,

Curtiss (5) has noted, under constant illumination, rhythms in the rate of transpiration of certain plants. A pronounced maximum occurs near midday, with other minor oscillations. He has further noted that the stomata are more responsive to stimuli in the morning than in the afternoon.

From the foregoing account of earlier work it is seen that in all cases when plants are exposed to the normal alternation of darkness and light a regular daily "periodicity" is thus induced; and that when these conditions are rendered uniform this "periodicity" is gradually lost. From the work of Kellicott (14), Karsten (12), and from the results of the present paper, it is seen that there is present, under uniform conditions, a "rhythm" which is entirely independent of the "periodicity" induced by environmental changes. This rhythm is concealed by the more prominent periodicity under normal conditions. Previous workers, including both Kellicott and Karsten, have failed to point out this difference. It is the object of this work to determine to what extent these rhythms are present in other species than those mentioned above, their probable cause, and their relation to the time of day.

Materials and Methods

Materials

For the present study the following materials were used: radicles from seedlings of *Cucurbita Pepo* L., *Lupinus albus* L., *Pisum sativum* L., *Vicia faba* L., *Allium Cepa* L., and *Zea everta* Sturt.; and roots from germinating bulbs of *Allium Cepa* L., *A. canadense* L., and *A. cernuum* Roth.

Methods

Elongation. Seeds or bulbs were germinated in moist sawdust loosely packed in glass germinating chambers. These chambers had one face ground plane and polished, and measured 75 x 100 x 400 mm. The plane face was ruled in horizontal lines 2 mm. apart. Germination, except in a few cases, was secured at temperatures constant to within one degree C., though the temperatures used in different series ranged from 22° to 26° C. The cultural chambers were kept tilted a few degrees from the vertical while in the incubators, in order to have the root tips always growing directly along the inside of the chamber face. When observations were to be made, the chambers were taken from the incubators and placed before a horizontal microscope fitted with an eye-piece micrometer. The exact position of the tip of the growing root was then determined by measuring the number of micrometer spaces between it and the horizontal lines (on the face of the chamber) below and above it. In this way the exact position of the tip of the root was determined every hour throughout the course of the experiment, and the increments of growth calculated from the changes in this position. Since one eye-piece (micrometer) division was equal to 0.04 mm. absolute measurement, the growth increments could be measured accurately to 0.01 mm.

Cell Division. Root tips of the species to be studied were cut [illegible] seedlings (or germinating bulbs) germinated at 22° to 26° C. (but al[illegible] constant to within one degree for any particular series) in moist sawd[illegible] ordinary 4-inch pots. These tips were cut at intervals of two hours, [illegible] 96 hours after the seeds or bulbs had been placed in the germinating [illegible] The tips were fixed 24 to 36 hours in medium chrom-acetic solution, wa[illegible] dehydrated, imbedded in paraffin in the usual way, cut into sections [illegible] microns in thickness, and stained in Delafield's haematoxylin. Only those slides showing sections cut exactly parallel to the long axis of the tip were used. Two or three slides were chosen for each hour, and from each slide chosen the median section and one on either side were marked off. The slides, having been previously labeled with a writing diamond, were now given a new number without regard to the first one, and all counting of dividing cells was done by this last number. The two numbers were not compared until the entire series had been counted, so that any influence due to a knowledge of the time of day of the particular slide being counted was avoided.

A typical observation. The slides and particular sections having been chosen for observation, the diameter of the section was then measured at a point where the root had attained a uniform diameter. Measurements were made by the eye-piece micrometer scale and are given accurate to the nearest 0.0085 mm. The diameter measured, the slide was moved by the mechanical stage to a point at a distance from the growing point of the tip equal to twice the diameter (measured where the root had attained uniform diameter) of the tip. The number of dividing cells in this area between the growing point and the imaginary line drawn across the section was then carefully counted. In order to facilitate the counting, a small rectangle was made by gluing four straight bristles (one for each side of the rectangle) into the eye-piece of the microscope. The section was then moved back and forth through this rectangle for counting. The number of cells dividing were recorded under the four phases: prophase, metaphase, anaphase, and telophase. All cells with nuclei between an evident spirem and the completion of the cell plate in the telophase were considered to be dividing. The area of the field observed was then determined by carefully counting the number of squares of a net eye-piece micrometer necessary to cover the field. This area was reduced to absolute measurement in square millimeters. It was soon discovered that the value so obtained very nearly approximated the value $7d^2/4$, where d equals the diameter of the section in millimeters. The amount of difference between the two methods mentioned above was always very small and constant for a given species. All calculations of areas given below were made from the latter formula.

Since it has been shown by a number of investigators, among whom are Amelung (1), Sanio (31), and, more recently, La Rue and Barthett (18), that in corresponding organs of plants of the same species variation in cell

size is so slight that variations in size of the part are due almost entirely to differences in cell number, and not in cell size, the number of dividing cells in all cases was reduced to the proper proportion for a certain constant area of one square millimeter. This thus avoided error due to observation of roots of different sizes. This care was taken by Kelbcor; [illegible], but was omitted by Karsten (12 and 13). In all cases the area observed contained practically all of the dividing cells.

INVESTIGATION

Elongation

Pisum sativum. Seeds of two varieties, *viz.*, wrinkled (*gradus*) and smooth (No. 1 White Field of D. M. Ferry & Co.), were allowed to germinate, and when the radicles had attained a length of 50–70 mm observations began. All observations were made in a dark room and at constant temperatures, so that the results obtained could not have been influenced by environmental changes of temperature and illumination. In all the following plant and curve numbers it has been thought best to reproduce here the numbers as they actually occur in the original data. Space will permit the reproduction of but few of the mass of figures and curves upon which these results are based. Table 1 shows a representative set of elongation measurements; while in table 2 the times of maxima and minima of ten plants out of a total of 50 of this species studied are grouped. The other 40 are duplicates of one or other of those given in this table.

A study of curves 193 and 194 (figures in table 1) shows that elongation is rhythmic or oscillatory in nature, three waves of elongation occurring in the 24-hour period. Elongation is least rapid at 1–3 P.M., rises to a maximum at 5–7 P.M., with other maxima at 11 P.M. to 1 A.M., and 5–7 A.M., and minima at 9 P.M. and 3–5 A.M. These plants were of the smooth-seeded variety. Curve 174 again shows three waves of elongation; here, however, the maxima occur at 11 A.M., 9 P.M., and 5 A.M., and the minima at 7 P.M., 1 A.M., and 7 A.M. Curve 160 also shows three waves, with maxima at 1 P.M., 11 P.M., and 5 A.M., and minima at 11 A.M., 9 P.M., and 3 A.M. Comparison of these curves seems to show little uniformity. They are, however, not comparable for two reasons: (1) the first two are obtained from plants of the smooth-seeded variety and the latter two are from those of the wrinkled-seeded variety; (2) germination[2] in the case of the first two was begun at 9 A.M., in no. 174 it was begun at 8 P.M., and in no. 160 at 6 P.M. In order to make no. 174 comparable, with respect to time after initiation of activity, to plants started at 9 A.M., it will be necessary to move the entire curve (no. 174) backward 11 hours or forward 13 hours; similarly, no. 160 will have to be moved backward 9

[2] In all cases throughout this paper the time stated for beginning of germination is the time when seeds were placed in the germinating chambers.

TABLE 1. *Pisum sativum. Elongation of Plants 193 and 194 (Smooth-seeded variety*

Time	Temp.	193		194	
10 A.M.	20.3	0.912		1.135	
11	20.8	0.955	1.867	1.045	2.180
12 M.	20.8	0.855		0.900	
1 P.M.	20.6	0.706	1.561	0.855	1.755
2	21.0	0.784		0.855	
3	21.0	1.116	1.900	0.765	1.620
4	21.0	1.180		1.045	
5	21.0	0.837	2.017	0.977	2.022
6	21.0	0.720		1.180	
7	21.2	0.675	1.395	1.135	2.315
8	21.0	0.315		0.900	
9	21.0	0.225	0.540	1.000	1.900
10	21.2	0.651		1.085	
11	21.0	0.457	1.108	0.865	1.953
12 N.	21.0	0.708		0.955	
1 A.M.	21.0	0.425	1.133	0.888	1.843
2	—	0.475		0.850	
3	21.0	0.475	0.950	0.850	1.700
4	21.0	0.750		0.600	
5	21.0	0.884	1.634	0.791	1.391
6	—	0.675		0.972	
7	21.0	0.675	1.350	0.972	1.944
8	—	0.585		0.522	
9	21.0	0.585	1.170	0.522	1.044

hours or forward 15 hours. The justification for this will be discussed later in connection with curves for cell division. The times of maxima and minima for plants given in table 2 are rewritten there on the basis of having started at 9 A.M. The lack of uniformity at first apparent disappears when

TABLE 2. *Pisum sativum. Grouping of Maxima and Minima of Elongation, Wrinkled-seeded Variety*

Plant	Maxima			Minima		
155	12 M.	10 P.M.	4 A.M.	10 A.M.	8 P.M.	2 A.M.
159	2 P.M.	10 P.M.	6 A.M.	10 A.M.	6 P.M.	4 A.M.
160	2 P.M.	8 P.M.	4 A.M.	12 M.	6 P.M.	2 A.M.
165	2 P.M.	8 P.M.	4 A.M.	10 A.M.	6 P.M.	2 A.M.
169	4 P.M.	8 P.M.	6 A.M.	12 M.	6 P.M.	4 A.M.
170	2 P.M.	10 P.M.	4 A.M.	12 M.	8 P.M.	6 A.M.
174	6 P.M.	12 N.	10 A.M.	2 P.M.	8 P.M.	8 A.M.
175	10 P.M.	12 N.	10 A.M.		4 P.M.	2 A.M.
	2 P.M.	12 N.	6 A.M.	8 A.M.	8 P.M.	12 N.

SUMMARY

Maxima.....2–6 P.M. 8–12 P.M. 4–6 (10)[3] A.M.
Minima.....10 A.M.–2 P.M. 6–8 P.M. 2–4 (6)[3] A.M.

Smooth-seeded Variety

Plant	Maxima			Minima		
193	5 P.M.	1 A.M.	5 A.M.	1 P.M.	9 P.M.	3 A.M.
194	7 P.M.	11 P.M.	7 A.M.	3 P.M.	9 P.M.	5 A.M.

[3] Parentheses indicate an occasional variation in time to that enclosed by them.

the curves are plotted on an equal basis with respect to time after initiation of activity. Thus in general, in table 2, maxima occur at 2–6 P.M., 8–12 P.M., and 4–6 (10) A.M. in the wrinkled-seeded variety; and at 5–7 P.M., 11 P.M.–1 A.M., and 5–7 A.M. in the smooth-seeded variety; while the minima occur at 10 A.M.–2 P.M., 6–8 P.M., and 2–4 (6) A.M.; and 1–3 P.M., 9 P.M., and 3–5 A.M. respectively. It will be seen that the general character of the curves is the same for both wrinkled-seeded and smooth-seeded varieties. Both exhibit three waves of elongation in the 24-hour period, though the precise time of maxima and minima is usually slightly later in the smooth-seeded than in the wrinkled-seeded variety.

Except in a few cases, observations ceased at the close of the 24-hour period. In those few cases in which observations continued longer there was no material difference between the two days. The curve continued in the same oscillatory or rhythmic manner. The outstanding feature of these results is the rhythmic nature of elongation.

Lupinus albus. Seeds were germinated, and seedlings studied, in the same manner as above described for Pisum. In table 3 the maxima and minima of eight representative curves are grouped. A total of 23 different individuals was studied. It will be seen that here again three waves of elongation occur in the 24-hour period, with maxima at 1–3 P.M., 7 P.M.–1 A.M., and 5–7 A.M.; and minima at 11 A.M.–1 P.M., 5–9 P.M., and 3–5 A.M. Germination was begun at 9 A.M.

TABLE 3. *Lupinus albus. Grouping of Maxima and Minima of Elongation*

Plant	Maxima			Minima		
68...	3 P.M.	11 P.M.	7 A.M.	1 P.M.	9 P.M.	5 A.M.
69...	1 P.M.	1 A.M.	7 A.M.	11 A.M.	7 P.M.	5 A.M.
70...	3 P.M.	11 P.M.	7 A.M.	1 P.M.	9 P.M.	3 A.M.
71...	3 P.M.	7 P.M.	5 A.M.	1 P.M.	5 P.M.	3 A.M.
72...	1 P.M.	9 P.M.	7 A.M.	1 P.M.	7 P.M.	5 A.M.
73...	3 P.M.	11 P.M.	7 A.M.	1 P.M.	5 P.M.	3 A.M.

SUMMARY

Maxima.....	1–3 P.M.	7 P.M.–1 A.M.	5–7 A.M.
Minima.....	11 A.M.–1 P.M.	5–9 P.M.	3–5 A.M.

Curves 70 and 73 illustrate the character of elongation in two of these plants. While the corresponding waves (in regard to time of occurrence) in the various plants are not all of the same amplitude, the times of their maxima and minima are very close, and the character of the curves is very similar, indicating that once these activities are initiated they proceed in rhythmic fashion; and the time interval of the waves is a more or less nearly constant feature. The only earlier work on the root of Lupinus is that of Strehl (36). His results are not comparable with those of the present paper since his seedlings were exposed to the alternation of day and night, and hence any oscillations not induced by this alternation would be likely to be entirely concealed by the more prominent daily periodicity.

Allium Cepa. Roots from both germinating seeds and bulbs were used. The bulbs were uniform and of a medium-sized white variety, and the seeds of the Yellow Danvers (D. M. Ferry & Co.) variety.

Roots from Bulbs. In table 4 are grouped the times of maxima and minima of the elongation of the roots of seven different plants. These are chosen to represent the various types of curves, and consequently show somewhat less approach to uniformity than when all curves are considered. Curves 272 and 296 show three waves of elongation in the 24-hour period. The maxima come at 7–9 A.M., 7 P.M., and 1 A.M.; and the minima at 1–3 P.M., 11 P.M., and 5 A.M. This type of curve is exhibited by about

TABLE 4. *Allium Cepa (bulb). Grouping of Maxima and Minima of Elongation*

Plant	Maxima				Minima			
263....	11 A.M.		1 A.M.		9 P.M.		3 A.M.	
261....	7 A.M.	1 P.M.	9 P.M.	1 A.M.	9 A.M.	5 P.M.	11 P.M.	5 A.M.
264....	11 A.M.	5 P.M.	9 P.M.	5 A.M.	3 P.M.	7 P.M.	11 P.M.	7 A.M.
254....	9 A.M.	9 P.M.	1 A.M.		3 P.M.	11 P.M.	7 A.M.	
271....	11 A.M.	7 P.M.	3 A.M.		5 P.M.	11 P.M.	5 A.M.	
272....	9 A.M.	7 P.M.	1 A.M.		3 P.M.	11 P.M.	5 A.M.	
296....	7 A.M.	7 P.M.	1 A.M.		1 P.M.	11 P.M.	5 A.M.	

SUMMARY

Maxima.....7–11 A.M. 7–9 P.M. 1–3 (5) A.M.
Minima.....1–5 P.M. 9–11 P.M. 3–7 A.M.

75 percent of the plants. Comparison with Kellicott's (14) curves shows only slight differences in the exact time of occurrence of maxima and minima. A second type of behavior is illustrated in curves 261 and 264 where four waves are found in the 24-hour period. Three of these waves correspond closely, in regard to time, to those of the other plants which show three waves. A third type of curve is that shown by plant 263 where but two waves are found in the 24-hour period. Kellicott (14, page 545, fig. 7, curve II) shows a similar curve with but two waves. Two plants out of a total of 50 showed this type of curve.

Roots from Seeds. Curves for elongation of roots from seedlings differ from those from bulbs mainly in that they are about equally divided between three- and four-wave types. In curves 275 and 288 three waves are shown, while curves 273, 274, and 276 exhibit four waves. All of these observations were made under identical conditions. Plants 275 and 276 grew beside each other in the same culture chamber, and a study of their curves shows how similar a four-wave curve is to one of three waves. It will be seen that the noon maximum comes two hours earlier in 276 than in 275, while the afternoon minimum comes two hours later in 276 than in 275. The other maxima, common to both, coincide; the difference in number of waves being due to the fact that 276 reaches its third maximum much earlier, sinks to a minimum, and then rises to a fourth maximum by the time 275

TABLE 5. *Allium Cepa (Seed). Grouping of Maxima and Minima of Elongation. Four-Wave Type*

Plant	Maxima			Minima			
276	5 A.M.	11 A.M.	7 P.M.	1 A.M.	7 A.M.	5 P.M.	9 P.M.	3 A.M.
277	9 A.M.	1 P.M.	7 P.M.	5 A.M.	11 A.M.	5 P.M.	1 P.M.	7 A.M.
278	9 A.M.	1 P.M.	7 P.M.	1 A.M.	11 A.M.	5 P.M.	9 P.M.	3 A.M.
279	9 A.M.	1 P.M.	7 P.M.	3 A.M.	11 A.M.	3 P.M.	9 P.M.	7 A.M.
283	9 A.M.	1 P.M.	7 P.M.	3 A.M.	11 A.M.	3 P.M.	9 P.M.	7 A.M.

SUMMARY

Maxima.....5–9 A.M. (11 A.M.) 1–3 P.M. 7 P.M. 1–5 A.M.
Minima.....(7) 11 A.M. 3–5 P.M. 9 P.M. 11 A.M.–3 7 A.M.

has attained its third maximum. A similar comparison of curves 281 and 283 shows again how similar in general character are the curves of the two types. In curve 276 the extra wave appears in the hours just preceding and just following midnight, while in 283 the extra wave is only a very low-crested one and appears during the forenoon. In table 5 the maxima and minima of five different curves of the four wave type are grouped. It is seen that these curves are very similar and that there is very little overlapping of times of maxima and minima. In table 6 the summary of these

TABLE 6. *Allium Cepa (Seed). Comparison of Maxima and Minima of Elongation in Four-wave Curves with those of Three Waves*

Maxima

Four-wave type See table 5	5–9 A.M.	11 A.M.–3 P.M.	7 P.M.	1–5 A.M.
Three-wave type				
275	5 A.M.	1 P.M.	7 P.M.	
280		11 A.M.	5 P.M.	3 A.M.
286	5 A.M.	11 A.M.	7 P.M.	
288	7 A.M.		5 P.M.	11 P.M.
281	5 A.M.	9 A.M.	5 P.M.	

Minima

Four-wave type See table 5	(7) 11 A.M.	3–5 P.M.	9 P.M.–1 A.M.	3–7 A.M.
Three-wave type				
275		3 P.M.	1 A.M.	7 A.M.
280		1 P.M.	9 P.M.	7 A.M.
286		5 P.M.	9 P.M.	7 A.M.
288	9 A.M.		7 P.M.	5 A.M.
281		3 P.M.	11 P.M.	7 A.M.

curves is compared with five different curves of the three-wave type. It will thus be seen that the three-wave curves are, as individuals, very similar to those of the four-wave type, but differ among themselves primarily as to which of the waves (present in the four-wave curves) is omitted. The seeds for this work began germination at 9 A.M.

Cucurbita Pepo. Space will not permit so extensive a discussion as given above for Pisum, Lupinus, and Allium. Nothing unlike what we have already seen above was found in the study of this species. Curves

111 and 112, out of a total of 14 different plants studied, are given on Plate XXIV. In these, also, three waves of elongation occur in the 24-hour period.

Zea everta. For this study the White Rice (D. M. Ferry & Co.) variety was used. A single curve is shown on Plate XXIV for elongation. Too little work was done on this species to warrant definite conclusions. The curve, 102, shows two waves of elongation in the 24-hour period.

Summary for Elongation. Summarizing briefly in regard to elongation, we find that (1) elongation in all plants studied proceeds in a wave-like fashion, two to four waves being exhibited in the 24-hour period; (2) there is more or less variation among the various individuals of the same species in regard to the precise time of day of the occurrence of maxima and minima, though these can be arranged into definite groups which show very little overlapping of time (see tables 2–6); (3) it is indicated, though not definitely proven, in the case of Pisum, that the precise time of the occurrence of maxima and minima depends upon the time when germination was begun, and shows no relation to the actual time of day. This latter point will be taken up and definitely proven in connection with rhythms in cell division. This fact, if true, might also account for a great deal of the variation in elongation curves of plants of the same species placed in the germinating chambers at the same time, since it is possible that some of the seeds may have coats that are more permeable to water than others, and hence the precise time of initiation of metabolic activity would vary slightly.

Cell Division

Pisum sativum. For this work root tips from both the wrinkled-seeded and smooth-seeded varieties of peas were used. Curve 2 (figures in table 7) shows results obtained from a study of the wrinkled variety. Seeds were placed in germinating pots at 9 A.M. at a temperature of 25° C. and allowed to germinate for 72 hours. The radicles had attained a length of 20–50 mm. when killing and fixing began. It will be seen that three waves of cell division occur in the period of 24 hours. The three maxima come at 1 P.M., 5 P.M., and 5 A.M.; and the minima come at 11 A.M., 3 P.M., and 9 P.M. The two maxima coming at 5 P.M. and 5 A.M. are about equal in extent. It will be noticed throughout the curves that follow that those waves in the various curves from roots of the same variety of seed which are coordinate in regard to time of appearance, are not always of the same amplitude. Kellicott (14) found similar results in *Podophyllum peltatum.* A study of the figures from which this curve is drawn (table 7) shows remarkable uniformity of the different roots for the same hour. Only at 5 and 11 A.M. do any appreciable differences occur, and then they are of such a nature that they do not affect the character of the curve. Curve 27 shows results from a similar study of the smooth-seeded variety. These seeds

TABLE 7. *Pisum sativum. Wrinkled-seeded Variety. Figures for Cell Division, Curve 2. Germination began at 9 A.M. January 21-25, 1913*

Time	Temp.	Diam. Area	Dividing Cells				Total	Diam. Area	Area Tip	Area Tip
			Pro.	Meta.	Ana.	Tel.				
9 A.M...	25.0	.748[4]	160	47	11	34	252	257		
		.977[5]	180	49	8	36	273	279	284	
			233	47	7	23	310	317		
		.935	262	63	8	35	368	240		
		1.529	239	52	13	44	348	227	241	259
			263	66	10	55	394	257		
		.748	190	45	8	25	268	274		
		.977	147	40	9	32	228	233	252	
			166	43	5	32	246	251		
11 A.M...	25.0	1.03	107	42	3	9	161	87		
		1.856	108	37	4	14	163	88	98	
			168	38	4	15	225	121		
		.858	112	50	12	46	220	168		
		1.188	83	25	7	35	150	115	138	130
			98	40	10	28	176	135		
		.901	188	61	6	33	288	203		
		1.421	132	55	6	30	223	157	180	
1 P.M...	26.0	.935	249	50	16	37	352	230		
		1.529	351	61	12	32	456	301	278	
			320	51	9	30	410	272		
		.867	327	39	5	29	400	304		
		1.315	320	43	11	38	412	313	319	341
			370	43	6	25	444	337		
		8.42	306	62	13	66	447	361		
		1.237	288	58	13	55	414	334	339	
			272	75	8	45	400	323		
3 P.M...	26.0	.875	221	34	4	29	288	214		
		1.340	288	36	12	22	358	265	240	
			239	42	17	27	325	242		267
		.859	292	51	14	28	385	295		
		1.290	307	54	9	28	398	305	294	
			285	44	10	32	371	284		
5 P.M...	25.5	.988	516	87	14	62	679	396		
		1.707	598	62	11	50	721	421	405	
			519	80	31	66	696	406		
		.918	507	46	10	56	619	419		
		1.475	412	59	9	41	521	352	397	397
			374	59	16	38	487	330		
		.825	368	63	10	46	487	408		
		1.189	368	67	18	33	486	408	419	
			414	54	12	44	524	439		

[4] Diameter of section in millimeters, always upper number.

[5] Area counted, see page 386.

[6] C = Constant necessary for reduction of figures to common area of 1 sq. mm.

TABLE 7 (*Continued*)

Time	Temp.	Diam. Area	Dividing Cells				Total	Total × C	Ave. 1 Tip	Ave. 3 Tips
			Pro.	Meta.	Ana.	Telo.				
7 P.M.	25.25	.867	375	68	15	64	522	397		
		1.315	367	83	11	55	516	392	403	
			387	88	19	60	554	421		
		.782	325	49	8	49	431	402		
		1.070	328	41	9	33	411	384	375	368
			278	43	8	33	362	338		
		.850	282	64	14	51	411	326		
		1.261	324	62	11	89	456	361	327	
			242	62	15	53	372	295		
9 P.M.	25.25	.833	156	52	11	34	253	208		
		1.212	182	44	7	40	273	225	226	
			191	55	12	40	298	246		
		.910	204	49	11	43	307	211		
		1.448	178	57	11	47	293	203	204	237
			170	54	14	47	285	197		
		.842	237	65	10	36	348	281		
		1.237	256	66	10	30	362	292	280	
			227	67	6	32	332	268		
11 P.M.	25.0	.884	301	56	9	30	396	289		
		1.368	309	43	21	35	408	298	284	
			277	49	13	25	364	266		
		.774	231	56	7	42	336	321		
		1.047	231	61	12	38	341	325	316	271
			200	63	17	38	318	303		
		.833	148	42	7	22	219	180		
		1.212	196	33	12	37	278	229	213	
			189	37	8	45	279	230		
1 A.M.	25.0	.816	255	55	12	40	362	311		
		1.165	326	64	11	38	349	299	315	
			273	65	10	45	393	337		
		.842	274	60	10	37	381	308		
		1.237	274	59	10	56	399	322	316	295
			260	70	7	57	394	318		
		.910	246	59	8	48	361	249		
		1.448	257	85	6	23	371	256	256	
			271	58	18	34	381	263		
3 A.M.	25.0	.979	382	53	5	15	455	272		
		1.677	322	37	12	24	395	237	265	
			376	57	8	30	472	276		
		.807	343	45	10	30	428	375		
		1.140	291	56	12	24	383	336	365	301
			331	61	20	28	440	385		
		.884	355	40	4	31	653	314		
		1.369	258	38	9	20	023	237	272	
			270	45	10	39	44	266		

TABLE 7 (*Concluded*)

Time	Temp.	Diam. Area	Dividing Cells				Total	Total Vac.	Vac. in Tip	Vac. in Cyl.
			Pro.	Meta.	Ana.	Telo.				
5 A.M....	25.0	.988	445	53	15	36	549	319		
		1.707	319	52	12	47	430	251	279	
			345	66	19	30	460	268		
		.918	497	71	23	39	630	427		
		1.475	510	87	25	59	681	461	438	381
			455	89	23	66	633	428		
		.850	456	56	16	44	572	452		
		1.261	360	64	12	37	473	375	425	
			437	74	14	45	570	450		
7 A.M....	24.0	.808	275	50	11	52	388	340		
		1.140	311	60	12	38	421	309	341	
			258	53	13	33	357	313		
		.859	240	57	10	53	360	278		
		1.290	253	63	11	52	379	292	279	305
			238	65	2	40	345	266		
		.791	218	46	14	36	314	285		
		1.093	221	59	6	47	333	302	294	
			202	55	12	56	325	295		

were placed in germinating pots at 9 A.M. and incubated for 72 hours at a temperature of 22°–23° C. Here also it will be seen that three waves occur in the 24-hour period. The maxima come at 3 P.M., 9 P.M., and 1 A.M.; and the minima at 11 A.M., 7 P.M., and 11 P.M. A comparison of curves 2 and 27 shows that the first two maxima of curve 2 each come just eight hours earlier (or 16 hours later) than two of curve 27, while the third maximum departs somewhat from this time relation. A similar relation exists between the minima.

Let us now turn to evidence in support of the contention that the time of occurrence of maxima and minima is related to the time of initiation of activity and not to time of day. Curve 28 is the result obtained from root tips of the smooth-seeded variety grown at the same time and in the same incubator as those represented by curve 27, with the difference that the seeds for curve 28 were placed in the germinating pots at 2 P.M., instead of 9 A.M. of the same day. In curve 28 three marked maxima occur with a very small fourth. Omitting, for the present, this extra small wave, we find maxima occurring at 7 P.M., 3 A.M., and 7 A.M., and minima at 3 P.M., 11 P.M., and 5 A.M. Now it will be seen that these seeds were started to germinate just 5 hours later than those of curve 27. Since root tips were cut and fixed every two hours, a difference of precisely five hours would not appear in the curves as such, but rather as a four- or six-hour difference. Comparison of the two curves will show that the 7 P.M. maximum of curve 28 is just four hours later than the 3 P.M. maximum of curve 27; similarly, the 3 P.M. and 11 P.M. minima of curve 28 are just 4 hours later than the

11 A.M. and 7 P.M. minima of curve 27; while the 3 A.M. and 7 AM.. maxima, and the 5 A.M. minimum of curve 28 are each just 6 hours later than the corresponding maxima and minimum of curve 27. Thus the entire curve 27 is earlier than curve 28 by an amount of time equal to the difference in time between the beginnings of germination. As further evidence on this point, a third series of root tips were cut at the same time and under identical conditions. The seeds for this third series were placed in the germinating pots at 8 P.M. Curve 31 shows the results of this study. In curve 28 a fourth wave was merely indicated, while in curve 31 there are definitely and clearly four waves. It is seen that because of the difference between the times when seeds were placed in germinating pots there would be expected to be a difference of just eleven hours between the times of initiation of activity in curves 27 and 31, and six hours between curves 28 and 31. Table 8 shows the maxima and minima of these curves correlated in respect to time (after initiation of activity) of their occurrence.

TABLE 8. *Pisum sativum. Correlation of Maxima and Minima of Curves 27, 28, and 31*

27	28		31		
Germination Began at 9 A. M.	Germination at 2 P. M.	Diff. from 27; 5 Hrs.	Germination at 8 P. M.	Diff. from 27; 11 Hrs.	Diff. from 28; 6 Hrs.
Maxima					
3 P.M.	7 P.M.	4	3 A.M.	12	8
9 P.M.	3 A.M.	6	7 A.M.	10	4
1 A.M.	7 A.M.	6	3 P.M.	14	8
	11 A.M.				
Minima					
11 A.M.	3 P.M.	4	9 P.M.	10	6
7 P.M.	11 P.M.	4	5 A.M.	10	6
11 P.M.	5 A.M.	6	11 A.M.	12	6
	9 A.M.				
			1 A.M.		

A study of this table shows that the same relation exists between curves 28 and 31, and 27 and 31, as is shown above between curves 27 and 28, *viz.*, there are in both curves 28 and 31 waves corresponding, in time after initiation of activity, to each of the three waves shown in curve 27. The extra (fourth) waves appearing in curves 28 and 31 not only do not have a corresponding wave in curve 27, but also seem not to be correlative to each other.

A further experiment of this same nature was carried out in which two series of peas of the smooth-seeded variety were placed in germinating pots at 9 A.M. and incubated at 24–25° C. for 48 hours. They were then removed from the incubators to a refrigerator where a recording thermometer showed the temperature to vary between 6.0° and −0.5° C. for a period of 48 hours. During the time of refrigeration, control plants were kept growing in the glass culture chambers used for elongation studies, and their elongation was measured. The elongation figures (omitted for lack of space) show that the temperature was sufficiently low to inhibit all but the slightest

activity. After the plants had been in the refrigerator for nine hours, and from that time until the end of the period of refrigeration, the amount of elongation of the individual plants ranged from 0.018 to 0.070 mm. per hour. In six hours after being taken from the refrigerator and incubated at a temperature of 24°–25° C. these same control plants had regained their normal rate of elongation for that temperature. At the end of the refrigeration period the seedlings from which root tips were to be cut were also incubated at a temperature of 24°–25° C. Series 33 (curve 33) was removed from the refrigerator at 9 A.M., and series 35 (curve 35) was removed at 1 P.M. A comparison of the two curves (table 9) shows that there are present, again,

TABLE 9. *Pisum sativum. Correlation of Maxima and Minima of Curves 33, 35, and 27*

33	35		27		
Removed from Refrigerator at 9 A. M.	Removed from Refrigerator at 1 P. M.	Diff. from 33	Germination Began at 1 A. M.	Diff. from 27	Diff. from 35
Maxima					
5 P.M.	9 P.M.	4	3 P.M.	2	6
11 P.M.	1 A.M.	2	9 P.M.	2	4
5 A.M.	9 A.M.	4	1 A.M.	4	8
Minima					
1 P.M.	7 P.M.	6	11 A.M.	2	8
9 P.M.	11 P.M.	2	7 P.M.	2	4
1 A.M.	5 A.M.	4	11 P.M.	2	6

three waves, and that the times of two of the maxima and one of the minima are just four hours later in curve 35 than in curve 33, while the 7 P.M. minimum of curve 35 is six, instead of four, hours later than the 1 P.M. minimum of curve 33; and that the 11 P.M. minimum and 1 A.M. maximum of curve 35 are each but two hours later than the corresponding minimum and maximum of curve 33. Hence, in general, these curves also differ from each other by a time interval equal to the difference in time between their initiation of activity after refrigeration.

A comparison of curves 33 and 27 (table 9) shows that with but one exception the maxima and minima of curve 33 occur just two hours later than the corresponding waves of curve 27. This exception is found where the 5 A.M. maximum of curve 33 comes four, instead of two, hours later than the 1 A.M. maximum of curve 27. While the particular amount of difference in time between waves in curves 33 and 27 has no special significance, the fact that the time interval between waves of one curve is the same as that between waves of the other curve, taken together with the relation we have just seen existing between all these other curves of Pisum, proves that these rhythms are regular and definite and not mere chance variations. It further indicates the truth of the contention that the time of occurrence of maxima and minima is related to the time of initiation of activity, and not to actual time of day.

We note from this study of cell division in Pisum that (1) once activity is

initiated it proceeds in a rhythmic fashion; (2) in general, three waves are shown in the 24-hour period; (3) the exact time of appearance of maxima and minima is dependent upon the time of initiation of activity and shows no relation to time of day.

Lupinus albus. Curves 1 and 13 show the results of a study of cell division in this species. These curves, again, show three waves. Curve 1 shows the first maximum and minimum coming about four hours earlier than the corresponding wave in curve 13, though the general character of the two curves is strikingly similar and their rhythmic nature is well demonstrated. It should be mentioned that the two curves were obtained from seeds of different lots. The seeds in both cases began germination at 9 A.M.

Allium Cepa, Roots from Bulbs. Curve 10 shows three waves of cell division with maxima coming at 1 P.M., 9 P.M., and 5 A.M.; and the minima at 3 P.M., 1 A.M., and 7 A.M. In comparing this curve with those given by Kellicott (14) it is found that the 1 P.M. maximum and the 3 P.M. and 7 A.M. minima correspond to maximum and minima at similar times in his curves; while the 9 P.M. maximum of curve 10 comes just two hours earlier than the 11 P.M. maximum of his curve I, and one hour later than the 8 P.M. maximum of his curve II (page 563 of his paper). The 1 A.M. minimum and 5 A.M. maximum of curve 10 find no equivalents in his curve I. In his curve III, however, a third maximum occurs at 5 A.M. It should be noted that no figures are given for 5 A.M. in his curve I, and hence it is possible that a third maximum may have been missed at this hour. Curve 24 is drawn from data obtained a year after that of curve 10, and from a different lot of bulbs. Other conditions were the same in both. In comparison it is seen that the noon maximum of curve 24 comes at 11 A.M. instead of 1 P.M.; the afternoon minimum comes at the same time as in curve 10; while an additional low-crested wave, with maximum at 5 P.M. and minimum at 7 P.M., appears between the times of the first and second waves of curve 10. The remaining waves are the same in both. Curve 24 thus shows four waves instead of the usual three. In comparing these curves with those of Kellicott's on Allium we note that the main difference is the larger number of waves here shown. Kellicott used much lower temperatures than those used in the present work, and it is possible that this may account for the smaller number of waves found in his curves.

Roots from Seeds. Curve 12 shows results from a study of roots from seeds of the Yellow Globe variety. It will be seen that there is little difference between this and curve 10 (from bulbs), three waves being found in each case. The essential difference is found in the fact that the curve does not drop so suddenly to a minimum after both the 1 P.M. and the 9 P.M. maxima, in curve 12, as does curve 10.

Zea everta. Curve 7 shows results obtained from a study of roots from seedlings of the White Rice variety. Germination began at 9 A.M. It will be seen that the curve is much more oscillatory in character. Karsten

(12) found much the same condition in *Zea Mays*. While the number of waves found in the 24-hour period is higher than in the case of any other species studied, yet the fact that mitotic activity proceeds in waves or rhythms is none the less clearly demonstrated.

Vicia faba. Curve 5 shows results obtained from a study of roots of *Vicia faba*. Germination began at 9 A.M. It will be seen that two waves of cell division occur in the 24-hour period. Maxima occur at 5 P.M. and 7 A.M. and minima at 1 P.M. and 1 A.M. Comparison of this curve with the figures given by Karsten (12, page 9) shows that he, too, found two extensive waves of cell division with maxima coming at 10 A.M. and 9 P.M., and minima at 4 P.M. and 7 A.M. Thus the maxima of curve 5 come just three and four hours earlier, and the minima three and six hours earlier, than in Karsten's results. Besides the two more extensive waves it will be seen that his figures show two very small waves, one coming in each larger wave. He, however, did not take into consideration variations in size of the sections counted, and this, taken together with a possible difference in time of beginning germination, probably accounts for the differences between his results and those of the present paper.

Allium cernuum. For this study bulbs were collected in the field in October, stored in boxes of soil, and kept in the open until ready for use the following January. Upon germination each bulb produced from two to four roots. Curve 23 shows results from this study; it will be seen that four very marked waves occur in the 24-hour period.

Allium canadense. For this study the small aerial bulblets were collected in October and stored in a dry, cool place until ready to be used the following January. Curve 22 shows results from this study. It will be seen that five waves of cell division occur in the 24-hour period.

A brief summary of the results obtained from this study of cell division shows the following facts: (1) the curve of cell division in all plants studied exhibits a number of oscillations in the 24-hour period, in the majority of plants three; (2) the exact time of occurrence of maxima and minima is dependent upon the time of initiation of activity and not on time of day.

Relation between Elongation and Cell Division

Historical

De Wildeman (39) has shown by exact measurements that cells of Spirogyra do not elongate during mitosis, while in the staminal hairs of Tradescantia there is very slight elongation of the cell during early prophases but none at all during the later stages. Ward (38) has shown in his study of cell division and elongation of filaments of *Bacillus ramosus* Fraenkel that elongation proceeds in a wave-like fashion and that "the period of cell division entails more or less cessation of growth." Kellicott (14) has shown that, in general, the same thing is true of elongation and cell division

in roots from bulbs of *Allium Cepa*, *i.e.*, the times of maxima of cell division are near the times of minima of elongation and *vice versa*. It should be noted that the observations of de Wildeman (39) and Ward (38) were made directly upon the dividing cell while it *was* dividing. The two processes were observed in one and the same cell. Such direct observation in the case of root tips is, of course, out of the question.

Experimental

Pisum sativum. In table 10 the times of maxima and minima of elongation and cell division in Pisum are compared. It is seen that in both the wrinkled-seeded and smooth-seeded varieties the times of maxima of elongation correspond very closely to the times of minima of cell division, and *vice versa*. A single exception is found in each variety: in the wrinkled-seeded variety the 11 A.M. minimum of cell division comes considerably later than the corresponding maximum of elongation in the majority of plants; and in the smooth-seeded variety the 11 A.M. minimum of cell division comes much earlier than the corresponding maximum of elongation. With the exception of this one divergence in each case there is a very close reciprocal relation existing between the rapidity of elongation and the number of cells undergoing division.

TABLE 10. *Comparison of Maxima and Minima of Elongation and Cell Division in Pisum*

WRINKLED VARIETY			
Elongation Maxima (see table 2)	2–6 P.M.	8–12 P.M.	4–6 (10) A.M.
Cell Division Minima (see curve 2)	3 P.M.	9 P.M.	11 A.M.
Elongation Minima	10 A.M.–2 P.M.	6–8 P.M.	2–4 (6) A.M.
Cell Division Maxima	1 P.M.	5 P.M.	5 A.M.
SMOOTH VARIETY			
Elongation Maxima (see table 2)	5–7 P.M.	11 P.M.–1 A.M.	5–7 A.M.
Cell Division Minima (see curve 27)	11 P.M.	11 P.M.	7 A.M.
Elongation Minima	1–3 P.M.	9 P.M.	3–5 A.M.
Cell Division Maxima	3 P.M.	9 P.M.	1 A.M.

TABLE 11. *Comparison of Maxima and Minima of Elongation and Cell Division in Lupinus*

Elongation Maxima (see table 3)	1–3 P.M.	7 P.M.–1 A.M.	5–7 A.M.
Cell Division Minima			
Curve 1	3 P.M.	1 A.M.	5 A.M.
Curve 13	7 P.M.	1 A.M.	7 A.M.
Elongation Minima	11 A.M.–1 P.M.	5–9 P.M.	3–5 A.M.
Cell Division Maxima			
Curve 1	9 A.M.	11 P.M.	3 A.M.
Curve 13	1 P.M.	11 P.M.	3 A.M.

Lupinus albus. In table 11 the maxima and minima of elongation and cell division in Lupinus are compared. It will be seen that here again there

is a very close reciprocal relation existing between elongation and cell division. A single large divergence occurs in the case of the 7 P.M. minimum of cell division in curve 13.

Allium Cepa. In table 12 the maxima and minima of elongation and cell division in *Allium Cepa* are compared. In the case of roots from bulbs we find, again, very nearly a reciprocal relation between rapidity of elongation and number of cells undergoing division. Another divergence is seen in the case of the 3 P.M. minimum of cell division in both curves 10 and 24 (or 7–9 P.M. maximum of elongation).

TABLE 12. *Comparison of Maxima and Minima of Elongation and Cell-Division in Allium Cepa*

Roots from Bulbs			
Elongation Maxima (see table 4)	7–11 A.M.	7–9 P.M.	1–3 (5) A.M.
Cell Division Minima			
Curve 10	7 A.M.	3 P.M.	1 A.M.
Curve 24	7 A.M.	3 P.M., 7 P.M.	1 A.M.
Elongation Minima	1–5 P.M.	9–11 P.M.	3–7 A.M.
Cell Division Maxima			
Curve 10	1 P.M.	9 P.M.	5 A.M.
Curve 24	11 A.M.–5 P.M.	11 P.M.	5 A.M.
Roots from Seeds			
Elongation Maxima (see table 5)	5–9 A.M.–(11 A.M.)	1–3 P.M.	7 P.M., 1–5 A.M.
Cell Division Minima			
Curve 12	7 A.M.	5 P.M.	3 A.M.
Elongation Minima	11 A.M.	3–5 P.M.	9 P.M., 3–7 A.M.
Cell Division Maxima	1 P.M.		9 P.M., 5 A.M.

In the case of roots from seeds all of the maxima and minima of cell division find corresponding minima and maxima respectively in elongation so that the reciprocal relation here is quite evident except for the extra fourth wave in elongation.

In general we may say that the times of maxima of elongation are near the times of minima of cell division and *vice versa* in all plants studied. This reciprocal relation is not so clearly expressed as in the case where both processes may be observed at the same time and in the same individual cell as Ward (38) found in *Bacillus ramosus* Fraenkel and de Wildeman (30) found in Spirogyra; but is probably as near as might be expected from the fact that the two processes must be observed, not only in different cells, but also in different individual roots.

DISCUSSION

The question naturally arises: What are the causes of the rhythm found both in the elongation and the cell division of the plants studied? That it may be due to external influences of changes in illumination and temperature

is out of the question, since this work was done in a dark room and the temperature was kept constant, except in a few cases, to within one degree. It seems quite clear, also, that it is not due to heredity, in the case of seedlings, as Semon (32) and Karsten (12) believed, since it has been shown by a number of earlier investigators that plants placed in continuous darkness and uniform temperatures gradually lose the periodicity which they had exhibited when exposed to the alternation of darkness and light. Now it would be expected that these rhythms would show some relation to the normal changes of night and day, even though the experimental plants were not so exposed, if the rhythms were due to the hereditary persistence of such effects upon the parent plants. It has been shown, however, in the case of *Pisum sativum* seedlings, that these rhythms have no relation to time of day, but rather that they depend, for the precise time of their appearance, upon the time of day when metabolic activity is initiated. It was at first thought that the rhythm might be due, in the case of germinating bulbs, to the persistence of a habit acquired by the bulb, while the bulb was itself growing and so exposed to the alternation of darkness and light, and the subsequent transfer of this habit to the growing parts. This is also disproved, since roots grown from seeds, in the case of *Allium Cepa*, exhibited the same rhythms as those grown from bulbs. That the rhythms of elongation and cell division may have a relation to the diurnal rhythms in atmospheric pressure and electrical potential is also out of the question, since it has been shown that the time of the waves in elongation and cell division depends upon the time of the initiation of metabolic activity, and that they vary according to the time when germination is begun, regardless of atmospheric conditions. Stoppel (34) found a relation existing between curves for sleep movements of plants and electrical potential of the atmosphere.

The two processes, growth and cell division, must necessarily go hand in hand as two of the vital activities of germinating seedlings. Just what the precise relation between them is, is not so definitely known, though it is quite evident that a certain size of the cell must be attained before cell division ensues, since cells from corresponding parts of different individuals of the same species vary but little in size. In a comparison of the curves for elongation and cell division it is seen that a general reciprocal relation exists between these two processes whereby there is a slowing-up in the rate of elongation at the time when there is the largest number of cells undergoing mitosis. The fact that the processes of elongation and cell division show such a reciprocal relation to each other within the individual cell is not so difficult to understand, since there is probably not enough energy available to permit both processes to go on at their maximum at the same time. It is to be recalled, however, that the zone of most extensive elongation in the root is not the same as the zone of mitotic activity (practically all mitoses occur within a zone bounded by the growing point and an imaginary line

across the section back from the growing point a distance equal to twice the diameter of the root). This reciprocal relation between elongation and cell division in the root as a whole might be explained on the same basis as that in the individual cell, provided there is a coordination within the root tip sufficient so that when a large number of cells are undergoing mitosis the total energy available within the tip is directed more to mitosis than toward growth and elongation, and hence the one process will be near its maximum when the other is near its minimum. Whether it be a matter of available energy or not, the fact remains that the two processes, elongation and cell division, do alternate with each other, both in the individual cell and in the root as a whole. Since neither process can go on for any considerable length of time to the exclusion of the other, the curve representing the extent of either will show waves such as those found in the present work. Thus, activity once initiated by the beginning of germination of the seed or bulb, these two processes, of necessity having a definite relation to each other, bring about the rhythms here found.

The fact that these rhythms have a definite interval in the various series of the same variety of seedlings, and that corresponding waves in the different series bear the same relation to each other as the time interval between the times of initiation of metabolic activity, *i.e.*, that the maxima and minima in the different curves depend for the time of their appearance upon the time when germination was begun, indicates that the ultimate cause of this alternation between mitosis and elongagion is entirely an internal cause and not related to external conditions and is in perfect accord with the above suggested energy hypothesis. This harmony in the various series of plants of the same variety shows, further, that the rhythms here found are not mere chance variations in activity which, when plotted, show such curves, but rather that the two processes, elongation and cell division, follow each other in a regular manner, the root tip being occupied with one and then with the other, and hence showing a regular and definite oscillation from the one to the other.

Whether or not this reciprocal relation existing between elongation and cell division is sufficient entirely to account for these rhythms, and whether there might not also be other rhythms independent of, and more or less confused with, these first rhythms, is a question not satisfactorily answered by the data at hand. The fact that the times of maxima of elongation in a few cases did not coincide with the times of the minima of cell division might seem to indicate that there were other factors influencing the course of these activities in the plant besides the alternation of elongation and cell division. It is conceivable that a relation might exist between growth activity (including mitosis) and available food supply, whereby these metabolic processes might, once initiated, gradually increase and finally outweigh the capacity of the enzymes to render stored food available. Then, with a lessening proportion of available food, a slowing down of these processes

must ensue until the food supply is again adequate, after which the same processes may be repeated. In other words, may there not be a certain inertia inherent in these vital processes, so that once they are in operation a certain force is required to check them, and, once slowed down, a certain force is again required to accelerate them? This might explain oscillations in either process independently of the other, or in the sum of the two processes, but it would not explain the reciprocal relation between the two processes. The possibility of growth rate exceeding that of enzymatic activity is apparent in the exhaustion effects found at higher temperatures in seedlings of *Zea Mays* and *Pisum sativum* by Lehenbauer (19) and Leitsch (20).

It is necessary, also, to distinguish between the terms "periodicity" and "rhythm." By "periodicity" the earlier workers meant a regular oscillation which was caused by the alternation of day and night or by other external changes, and which was lost when the environmental conditions were rendered constant; while the term "rhythm" in the present paper is restricted to mean any oscillation in activity which is definite and regular and not related to any external influences. Thus these roots in their development exhibit "rhythms" in the absence of changes in environment, but not a "periodicity" in the sense in which the older writers used the term.

Summary

1. Under constant uniform conditions elongation in all plants studied proceeds in a rhythmic manner, two or more waves occurring during the 24-hour period.

2. Nuclear and cell division proceed in a similar rhythmic fashion.

3. The times of occurrence of maxima and minima are dependent upon the time of initiation of metabolic activity and not upon the time of day by the clock.

4. Elongation and cell division, as regards time of maxima and minima, are, in general, reciprocals of each other.

5. This reciprocal relation existing between elongation and cell division accounts for a large share, at least, of the rhythms found in these plants.

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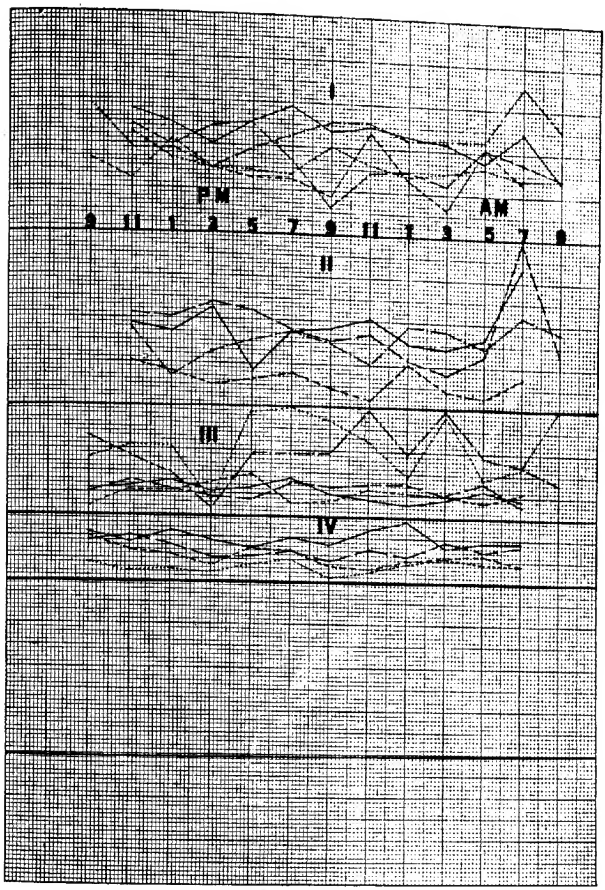
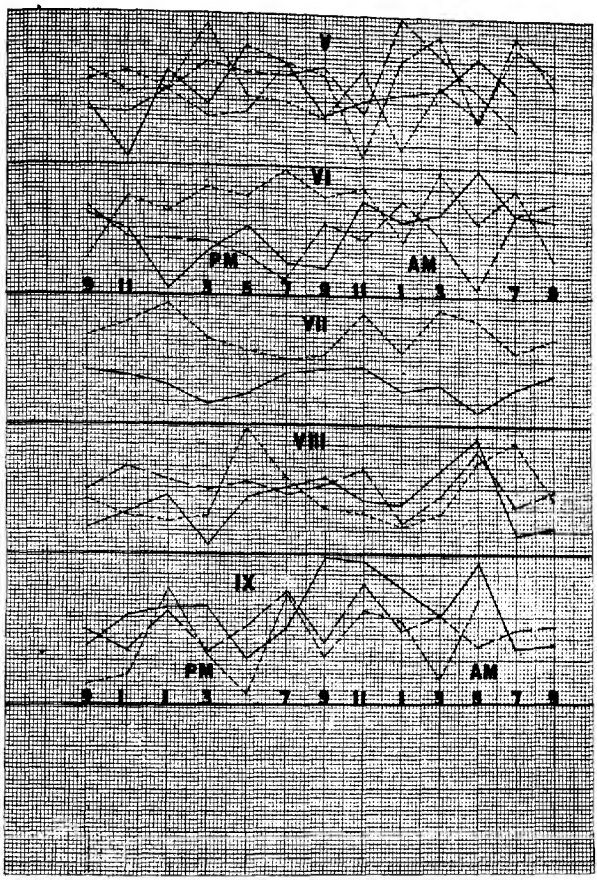


FRIESNER: DAILY RHYTHMS OF ELONGATION AND CELL DIVISION IN ROOTS.



FRIESNER: DAILY RHYTHMS OF ELONGATION AND CELL DIVISION IN ROOTS.

EXPLANATION OF PLATES XXIV AND XXV

rdinates in curves I–IV show rate of elongation in mm.,
th show the number of cells per sq. mm. undergoing mitosis.
o ly by the clock.

I

(Base line = 0. Scale, 1 square = .08 mm.)

3. (————) Elongation of *Pisum sativum*. Germination at 9 A.M.
04. (— — —) " " " " " " "
60. (– – – –) " " " " " " 6 P.M.
174. (·····) " " " " " " 8 P.M.
102. (— · —) " " *Zea everta*.

II

(Base line = 0. Scale, 1 square = .08 mm.)

70. (— — —) Elongation of *Lupinus albus*. Germination at 9 A.M.
73. (————) " " " " " "
272. (– – – –) " " *Allium Cepa* (bulb) " " "
296. (— · —) " " " " " " "

III

(Base line = 0. Scale, 1 square = 0.1 mm.)

275. (————) Elongation of *Allium Cepa* (seed). Germination at 9 A.M.
276. (— — —) " " " " " " "
288. (– – – –) " " " " " " "
111. (— · —) " " *Cucurbita Pepo* " " "
112. (·····) " " " " " " "

IV

(Base line = 0. Scale, 1 square = .08 mm.)

273. (————) Elongation of *Allium Cepa* (seed). Germination at 9 A.M.
274. (— — —) " " " " " " "
281. (– – – –) " " " " " " "
283. (·····) " " " " " " "

V

(Base line = 125. Scale, 1 square = 10 cells.)

2. (————) Mitosis in *Pisum sativum* (wrinkled) Germination 9 A.M.
27. (— — —) " " " " (smooth) " "
28. (– – – –) " " " " " " 2 P.M.
31. (·····) " " " " " " 8 "

VI

(Base line = 125. Scale, 1 square = 15 cells.)

33. (————) Mitosis in Pisum (smooth). From refrigerator at 9 A.M.
35. (— · — ·) " " " " " " 1 P.M.
7. (– – – –) " " *Zea everta*. Germination at 9 A.M.

VII

(Base line = 0. Scale, 1 square = 5 cells.)

1. (————) Mitosis in *Lupinus albus*. Germination at 9 A.M.
13. (– – – –) " " " " " " "

VIII

(Base line = 50. Scale, 1 square = 10 cells.)

10. (————) Mitosis in *Allium Cepa* (bulb). Germination at 9 A.M.
24. (— — —) " " " " " " "
5. (– – –) " " *Vicia faba*. Germination at 9 A.M.

IX

(Base line = 300. Scale, 1 square = 10.)

12. (————) Mitosis in *Allium Cepa* (seed). Germination 9 A.M.
22. (— — · –) " " *Allium canadense* " "
23. (– – – –) " " *Allium cernuum* " "